

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



B2

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68		(11) International Publication Number: WO 99/60164
A1		(43) International Publication Date: 25 November 1999 (25.11.99)
(21) International Application Number: PCT/US99/11066 (22) International Filing Date: 14 May 1999 (14.05.99) (30) Priority Data: 60/085,673 15 May 1998 (15.05.98) US (71) Applicant: QUARK BIOTECH, INC. [US/US]; 1059 Serpentine Lane, Pleasanton, CA 94566 (US). (72) Inventors: EINAT, Paz; Neve Nir 1/27, Ness-Ziona (IL). MOR, Orna; Emek Ayalon Street 12, Ganei Ilan, Kiryat Ono (IL). SKALFITER, Rami; Habanim Street 117/10, Ness-Ziona (IL). FEINSTEIN, Elena; Hahagana Street 12/29, Rehovot (IL). FAERMAN, Alexander; Haellah 2024/36, Bnei Aiish (IL). (74) Agents: KOWALSKI, Thomas, J. et al.; Frommer Lawrence & Haug LLP, 745 Fifth Avenue, New York, NY 10151 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MECHANICAL STRESS INDUCED GENES, EXPRESSION PRODUCTS THEREFROM, AND USES THEREOF		
(57) Abstract <p>This disclosure relates to osteoporosis. Moreover, the disclosure relates to mechanical stress induced genes, probes therefor, tests to identify such genes, expression products of such genes, uses for such genes and expression products, e.g., in diagnosis (for instance risk determination), treatment, prevention, or control, of osteoporosis or factors or processes which lead to osteoporosis; and, to diagnostic, treatment, prevention, or control methods or processes, as well as compositions therefor and methods or processes for making and using such compositions.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF THE INVENTION***MECHANICAL STRESS INDUCED GENES,
EXPRESSION PRODUCTS THEREFROM,
AND USES THEREOF*****5 RELATED APPLICATION**

This application is based upon and claims priority from U.S. Provisional application Serial No. 60/085,673, filed May 15, 1998.

Reference is also made to U.S. Provisional application Serial No. 60/084,944, filed May 11, 1998, and the full U.S. utility application, Serial No. _____, filed
10 May 11, 1999, naming as inventors Paz Einat, Rami Skaliter, Orna Mor and Sylvie Luria and assigned to the assignee of the present application (Kohn & Associates Attorney Docket No. 0168.00060), and claiming priority from U.S. Provisional application Serial No. 60/084,944 (herein "the May 11, 1999 Einat et al. full U.S. utility application").

15 U.S. Provisional application Serial No. 60/085,673, filed May 15, 1998, U.S. application Serial No. 60/084,944, and the May 11, 1999 Einat et al. full U.S. utility application, as well as each document or reference cited in that application, is hereby expressly incorporated herein by reference. Documents or references are also cited in the following text, either in a Reference List before the claims, or in the text itself;
20 and, each of these documents or references ("herein-cited documents or references"), as well as each document or reference cited in each of the herein-cited documents or references, is hereby expressly incorporated herein by reference. It is explicitly stated that the inventive entity of the May 11, 1999 Einat et al. full U.S. utility application is not another or others as to the inventive entity of this application; and, that the
25 inventive entity of the present application is not another or others as to the inventive entity of the May 11, 1999 Einat et al. full U.S. utility application.

FIELD OF THE INVENTION

This invention relates to osteoporosis. Moreover, the invention relates to mechanical stress induced genes, probes therefor, tests to identify such genes,
30 expression products of such genes, uses for such genes and expression products, e.g., in diagnosis (for instance risk determination), treatment, prevention, or control, of osteoporosis or factors or processes which lead to osteoporosis; and, to diagnostic,

treatment, prevention, or control methods or processes, as well as compositions therefor and methods or processes for making and using such compositions.

The present application also relates to a method for identifying genes that are regulated at the RNA level. More specifically, the present application relates to the rapid isolation of differentially expressed or developmentally regulated gene sequences through analysis of mRNAs obtained from specific cellular compartments. By comparing changes in the relative abundance of the mRNAs found in these compartments occurring as a result of application of a cue or stimulus to the tested biological sample, genes that are differentially expressed can be characterized.

The present invention especially relates to such methods with respect to bone cells and/or the stimulus being mechanical stress.

These and other areas to which the invention relates will be apparent from the following text.

BACKGROUND OF THE INVENTION

Bone is composed of a collagen-rich organic matrix impregnated with mineral, largely calcium and phosphate. Two major forms of bone exist, compact cortical bone forms the external envelopes of the skeleton and trabecular or medullary bone forms plates that traverse the internal cavities of the skeleton. The responses of these two forms to metabolic influences and their susceptibility to fracture differ.

Bone undergoes continuous remodeling (turnover, renewal) throughout life. Mechanical and electrical forces, hormones and local regulatory factors influence remodeling. Bone is renewed by two opposing activities that are coupled in time and space (Parfitt 1979). These activities - resorption and formation - are contained within a temporary anatomic structure known as a bone remodeling unit (Parfitt 1981). Within a given bone remodeling unit, old bone is resorbed by osteoclasts. The resorbed cavity created by the osteoclasts is subsequently filled with new bone by osteoblasts, which synthesize the organic matrix of bone.

Peak bone mass is mainly genetically determined, though dietary factors and physical activity can have positive effects. Peak bone mass is attained at the point when skeletal growth ceases, after which time bone loss starts.

In contrast to the positive balance that occurs during growth, in osteoporosis, the resorbed cavity is not completely refilled by bone (Parfitt 1988). Osteoporosis, or porous bone, is a progressive and chronic disease characterized by low bone mass and

structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures of the hip, spine, and wrist (diminishing bone strength).

Bone loss occurs without symptoms. The Consensus Development Conference (Am J Med 94:646-50, 1993) defined osteoporosis as "a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture."

Common types of osteoporosis include postmenopausal osteoporosis; and senile osteoporosis, which generally occurs in later life, e.g., 70+ years; *see, e.g.*, U.S. Patent No. 5,691,153 and documents cited therein and during its prosecution, all incorporated herein by reference.

Osteoporosis is estimated to affect more than 25 million people in the United States (Rosen 1997); and, at least one estimate asserts that osteoporosis affects 1 in 3 women (Keen et al. 1997). However, life expectancy has increased, and in the western world, 17% of women are now over 50 years of age; and, a woman can expect to live a third (1/3) of her life after menopause. Thus, some estimate that 1 out of every 2 women and 1 out of 5 men will eventually develop osteoporosis; and, that 75 million people in the US, Japan and Europe have osteoporosis. The World Summit of Osteoporosis Societies estimates that more than 200 million people world-wide are afflicted with the disease. The actual incidence of the disease is difficult to estimate since the condition is often asymptotic until a bone fracture occurs. It is believed that there are over 1.5 million osteoporosis-associated bone fractures per year in the U.S. of which 300,000 are hip fractures that usually require hospitalization and surgery and may result in lengthy or permanent disability or even death. (*See* Spangler et al. "The Genetic Component of Osteoporosis Mini-review; <http://www.csa.com.osteointro.html>)

Further, there is a 20-30% mortality rate related to hip fractures in elderly women (U.S. Patent No. 5,691,153); and, it is reported that such a patient with a hip fracture has a 10-15% greater chance of dying than others of the same age. Further, it is reported that although men suffer fewer hip injuries than women, men are 25% more likely than women to die within one year of the injury. *See* Sprangler et al., *supra*. Also, about 20% of the patients who were living independently before a hip fracture still remain confined in a long-term health care facility one year later; and, the treatment of osteoporosis and related fractures can cost over \$10 billion annually.

Accordingly, osteoporosis is a major health problem in virtually all societies (Eisman 1996; Wark 1996; U.S. Patent No. 5,834,200 and the documents cited therein, being hereby incorporated herein by reference).

Treatment for osteoporosis helps stop further bone loss and fractures, such as
5 HRT (hormone replacement therapy), bisphosphonates, e.g., alendronate (Fosamax), as well as, estrogen and estrogen receptor modulators, progestin, calcitonin, and vitamin D.

While there may be numerous factors that determine whether any particular person will develop osteoporosis, a step towards prevention, control or treatment of
10 osteoporosis is determining whether one is at risk for osteoporosis. Genetic factors are said to play an important role in the pathogenesis of osteoporosis (Ralston 1997; *see also* Keen et al. 1997; Eisman 1996; Rosen 1997; Cole 1998, Johnston et al. 1995; Gong et al. 1996; Wasnich 1996 *inter alia*).

Some attribute 50-60% of total bone variation (Bone Mineral Density; BMD),
15 depending upon the bone area, to genetic effects (Livshits et al. 1996). However, up to 85%-90% of the variance in bone mineral density may be genetically determined.

For instance, as studies have shown from family histories, twin studies, and racial factors, there may be a predisposition for osteoporosis (*see, e.g.*, Jouanny et al. 1995; Garnero et al. 1996; Cummings 1996; Lonzer et al. 1996). Several candidate
20 genes may be involved in this, most probably multigenic process.

Association between vitamin D receptor gene (VDR) allelic variation and BMD has been suggested. Restriction fragment length polymorphisms (RFLPs) at the vitamin D receptor (VDR) gene locus have been recently correlated to bone mineral density (BMD) and rate of bone loss (*see, e.g.*, Tokita et al. 1996; Cole et al. 1998;
25 Eisman 1996; Keen et al. Ralston 1997; Fujita 1996; Houston et al. 1996; Riggs et al. 1995; Fleet et al. 1995; Krall et al. 1995).

Collagen type I alpha gene has been implicated (*see, e.g.*, Dalglish 1997; Pereira et al. 1995). The COL1A1 and COL1A2 genes encode type I collagen, a key bone protein, and, therefore, may play a role in the genetic control of bone mass.

30 Mutation of the estrogen receptor (ER) gene may be implicated in some cases of osteoporosis (polymorphism of the ER gene has been correlated with BMD in some populations) (*see* Sano et al. 1995; *see also* U.S. Patent No. 5,834,200). Interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-alpha) have also been

implicated in the pathogenesis of osteoporosis in recent studies. These proinflammatory cytokines induce both cyclooxygenase (COX) and nitric oxide synthase (NOS) with the release of prostaglandin (PG) and NO, respectively. Cytokines have been shown to be powerful regulators of bone resorption and formation, though under superior control from oestrogen/testosterone, parathyroidhormone and 1,25(OH)2D3. Some of the cytokines primarily enhance osteoclastic bone resorption e.g. IL-1 (Interleukin-1), TNF (Tumor Necrosis Factor) and IL-6 (Interleukin-6), while others primarily stimulate bone formation e.g. TGF-beta (Transforming Growth Factor), IGF (Insulin-like Growth Factor) and PDGF (Platelet Derived Growth Factor).

There is need for clinical and epidemiological research to further explore and extend the current potential for practical control, prevention and treatment of the disease. A deeper knowledge of factors controlling bone cell activity and regulation of bone mineral and matrix formation and remodeling is desired.

Further, while certain genetic may be useful for detecting high bone mass or predisposition to low or high bone mineral density (*see* U.S. Patents Nos. 5,691,153 and 5,834,200), there is a need for further tests to determine risk for osteoporosis; and, there is a need for new treatments, preventatives, or means to control osteoporosis or factors or processes which lead to osteoporosis.

OBJECTS AND SUMMARY OF THE INVENTION

An object of the invention can include any one or any combination or all of: advancing clinical and epidemiological research and/or further exploring and extending the current potential for practical control, prevention and treatment of the disease; providing further knowledge of factors controlling bone cell activity and regulation of bone mineral and matrix formation and remodeling; providing further tests to determine risk for osteoporosis; and/or new treatments, preventatives, or means to control osteoporosis or factors or processes which lead to osteoporosis.

The present invention provides mechanical stress induced genes, probes therefor, a test to identify such genes, expression products of such genes, uses for such genes and expression products, e.g., in diagnosis (for instance risk determination), treatment, prevention, control, or osteoporosis or factors or processes which lead to osteoporosis. Thus, the invention further provides diagnostic, treatment, prevention, control methods or processes as well as compositions.

There is disclosed a method for identifying genes whose expression is regulated at the RNA level in an organism including the steps of selectively stimulating translation of an unknown target mRNA with a stress inducing element, the target mRNA being part of a larger sample of mRNA, dividing the sample of mRNA into pools of translated and untranslated mRNA and differentially analyzing the pools of mRNA to identify genes translationally regulated by the stress inducing element. The stress inducing element can include pathologic, environmental including chemical and physical stressors or other stimulus that induces mRNA translation. The stress inducing element can comprise mechanical stress. The sample can comprise bone cells that retain being bone cells in a culture, e.g., calvaria cells.

According to the present application, methods are disclosed for identifying genes that may be regulated on a number of possible regulatory levels. Such methods include the steps of exposing cells or tissue to a cue or stimulus such as mechanical, chemical, toxic, pharmaceutical or other stress, hormones, physiological disorders or disease; fractionating the cells into compartments such as polysomes, nuclei, cytoplasm and spliceosomes; extracting the mRNA from these fractions, and subjecting the mRNA to differential analysis using accepted methodologies, such as gene expression array (GEM).

For instance, the application discloses the use of RNA isolation from nuclei for isolating genes whose steady state levels show only minor changes, but which show high differential expression when detected by nuclear RNA probe. Most such genes are regulated at the transcriptional level. One type of regulation is shown using polysomes isolated from cells/tissues to identify genes whose mRNA steady state levels do not change, but are highly increased in the polysomes after application of a stress cue. Such genes are regulated strictly on the translation level. A subgroup of genes regulated on the translational level involves the existence of internal ribosome entry sites. A method is disclosed for identification of such genes, which includes inhibiting 5' cap-dependant mRNA translation in a cell, collecting a pool of mRNA from the cells, and differentially analyzing the pool of mRNA to identify genes with sequences coding for internal ribosome entry sites.

Thus, the application discloses a method or process for identifying genes whose expression is responsive to a specific cue or cues including the steps of:

(a) applying a cue to an organism or tissue or cells;

(b) isolating specific cellular fractions from the tissues or cells subjected to the cue;

(c) extracting the mRNA from the cellular fractions; and

(d) differentially analyzing the mRNA samples in comparison with control
5 samples not subjected to the cue to identify genes that have responded to the cue.

The cells or tissues can be bone cells which retain the nature of being bone cells when in a culture and the cue can be mechanical stress or a lack thereof.

The cue can be a toxin or a chemical, or a pharmaceutical, or a mechanical stress, or an electric current, or a pathogen or a pathological condition, or a hormone,
10 or a specific protein. The cue can be further defined as chemically treating the cells, or irradiating the cells, or depriving the cells of oxygen. The cue can be further defined as a stress-inducing element of unknown relationship to gene translation.

The genes can be identified at the translation level; genes regulated at the transcription level; genes regulated by RNA stability; genes regulated by mRNA
15 transport rate between the nucleus and cytoplasm; genes regulated by differential splicing; and genes regulated by antisense RNA.

The mRNA samples can be further fractionated into mRNA subfractions which are subjected to differential analysis to identify genes responsive to the cue at all levels of expression regulation as herein defined and to determine the abundance
20 and direction of the response. The mRNA sample can be fractionated into one or more subfractions from the group consisting essentially of cytoplasmic, nuclear, polyribosomal, sub polyribosomal, microsomal or rough endoplasmic reticulum, mitochondrial and spliceosome associated mRNA.

The differential analysis step can be selected from the group consisting of
25 differential display, representational differential analysis (RDA), suppressive subtraction hybridization (SSH), serial analysis of gene expression (SAGE), gene expression microarray (GEM), nucleic acid chip technology, oligonucleotide chip technology; DNA membrane arrays; direct sequencing and variations and combinations of these methods. The differential analysis step can be further defined
30 as identifying and measuring the genes regulated at the translation level. The differential analysis step can also be further defined as identifying and measuring the genes regulated at the transcription level. The differential analysis step can also be further defined as identifying and measuring the genes regulated by RNA stability.

The differential analysis step can additionally be further defined as identifying and measuring the genes regulated by mRNA transport rate between the nucleus and the cytoplasm. The differential analysis step can also be further defined as identifying and measuring the genes regulated by differential splicing. The differential analysis step additionally can be further defined as identifying and measuring the genes encoding secreted and membrane proteins. The differential analysis step can also be further defined as identifying and measuring the genes encoding for nuclear proteins.

The application further discloses a method for determining risk of developing a physiological or disease state based upon absence or decrease from normal cells of mRNA or protein from a gene shown to be down regulated in a mammal by an inventive or disclosed method comprising:

- (a) determining the level or status of mRNA in cells of said mammal; and/or
- (b) determining the level or status of corresponding protein in cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus the risk of developing a physiological or disease state.

The application further discloses a method for determining risk of physiological or disease state based upon presence or an increase from normal cells of mRNA or protein from a gene shown to be upregulated by an inventive or disclosed method in a mammal comprising:

- (a) determining the level or status of mRNA in cells of said mammal; and/or
- (b) determining the level or status of corresponding protein in cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a

transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a
5 presence or increase from normal cells of mRNA or protein and thus risk.

These "determining" methods can be diagnostic methods; e.g., methods for diagnosing a physiological or disease state.

The application further discloses a method for testing a medicament for or a gene therapy approach to a physiological or disease state or other factors causing or
10 contributing thereto or to symptoms thereof based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein of identified genes comprising an inventive or disclosed method additionally comprising: (a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or
15 increase from normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

The application still further discloses a method for treating, preventing or controlling a physiological or disease state comprising an inventive or disclosed method and additionally comprising administering a medicament or treatment therefor
20 or for a cause thereof or a symptom thereof.

The medicament or treatment can comprise the protein, a functional portion thereof, a vector expressing the protein or a functional portion thereof, or an inhibitor of the protein or of a functional portion thereof, or an inhibitor of a nucleic acid encoding the protein or a functional portion thereof.

25 Inventive or disclosed methods can further comprise:

- (d) determining the level or status of a second gene mRNA in cells of said mammal; and/or
- (e) determining the level or status of protein expressed by a second gene product in cells of said mammal; and
- 30 (f) comparing said level or status of that mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence,

which may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining risk.

- 5 Steps (a) and/or (b) and optionally (d) and/or (e) can be carried out *in vivo* and/or steps (a) and/or (b) and optionally (d) and/or (e) can be carried out *in vitro*. The determination in step (a) and optionally in step (d) can be effected by employing
- (i) a nucleic acid sequence corresponding to at least a part of the gene encoding at least part of the protein and optionally a second nucleic acid sequence corresponding
 - 10 to at least a part of the second gene encoding at least part of the second protein;
 - (ii) a nucleic acid sequence complementary to the nucleic acid sequence(s) of (i);
 - or
 - (iii) a primer or a primer pair hybridizing to the nucleic acid sequence(s) of (i) or (ii).

- 15 The determination in step (b) and optionally of step (e) can be effected by employing an antibody or a fragment thereof that specifically binds to the protein and optionally by employing a second antibody or a fragment thereof which specifically binds to the second protein.

- 20 In inventive methods, the stimulus can be mechanical stress or a lack thereof and the sample comprises bone cells which retain their characteristic thereof in cultures.

- The invention further provides a gene identification process comprising: preparation of probes from a model system; analysis of DNA chip hybridization; sequencing of clones showing differential expression; and optionally full-length
- 25 cloning of clones of interest.

The model system can comprise bone cells which retain their characteristic thereof in cultures which have mechanical stress or a lack thereof applied thereto. The bone cells can comprise a calvaria primary culture.

- 30 The invention further provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells of mRNA or protein from a gene shown to be down regulated by an inventive method comprising:

- (a) determining the level or status of mRNA in bone cells of said mammal; and/or
 - (b) determining the level or status of corresponding protein in bone cells of said mammal; and
 - (c) comparing said level or status of mRNA and/or protein with the corresponding
- 5 level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including
- 10 post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

The invention still further provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or other conditions involving mechanical stress or a lack thereof, based upon presence or increase from normal cells of mRNA or protein from

15 a gene shown to be upregulated by an inventive method in a mammal comprising:

- (a) determining the level or status of mRNA in bone cells of said mammal; and/or
 - (b) determining the level or status of corresponding protein in bone cells of said mammal; and
 - (c) comparing said level or status of mRNA and/or protein with the corresponding
- 20 level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity
- 25 of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells of mRNA or protein and thus risk.

The invention also provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to

30 osteoporosis or lower levels of osteoblasts and chondrocytes or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells of mRNA or protein from 608 comprising:

- (a) determining the level or status of mRNA in bone cells of said mammal; and/or

(b) determining the level or status of corresponding protein in bone cells of said mammal; and

- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells of mRNA or protein and thus risk.

Further still, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of imbalance as to osteogenic and chondrogenic cells or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein from 405 in a mammal comprising:

- (a) determining the level or status of mRNA in bone cells of said mammal; and/or
- (b) determining the level or status of corresponding protein in bone cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

Even further, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of being susceptible to environmental factors or other than genetic factors of osteoporosis or of predisposition of bones towards susceptibility to environmental factors, or less lymphoid cells, or osteoporosis, or other conditions involving mechanical stress or a lack thereof, based upon presence or

increase from normal cells or absence or decrease from normal cells of mRNA or protein from 274 in a mammal comprising:

- (a) determining the level or status of mRNA in bone cells of said mammal; and/or
- (b) determining the level or status of corresponding protein in bone cells of said
5 mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a
10 mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

These "determining" methods can be diagnostic methods; e.g., methods for
15 diagnosing osteoporosis or for diagnosing other conditions recited in the preamble of these "determining" methods.

Also, the invention further provides a method for testing a medicament for or gene therapy approach to osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving
20 mechanical stress or a lack thereof, based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein comprising a method according to any one of the foregoing inventive methods and additionally comprising:
(a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or increase from
25 normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

The invention also comprehends analogous methods with respect to other genes identified by inventive processes, e.g., CMF2-224, CMF2-45.

Similarly, the invention additionally provides a method for treating,
30 preventing or controlling osteoporosis or other conditions involving mechanical stress or a lack thereof, comprising a method according to any one of the foregoing inventive methods and further comprising administering a medicament or treatment for osteoporosis or a cause thereof or a symptom thereof.

Still further, the invention provides a composition comprising a gene or portion thereof or a protein or portion thereof expressed by the gene or portion thereof or an antibody or portion thereof which binds to the protein or portion thereof, wherein the gene is identified by an inventive method.

- 5 Even further still, the invention provides an osteoporosis or mechanical stress or lack thereof model comprising bone cells which retain their characteristic thereof in culture with mechanical stress applied thereto or an absence of mechanical stress applied thereto.

- 10 The invention additionally provides an isolated nucleic acid molecule encoding the herein identified protein 608 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

Further, the invention provides an isolated nucleic acid molecule encoding the herein identified protein 405 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

- 15 Also, the invention provides an isolated nucleic acid molecule encoding the herein identified protein 274 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

- 20 The invention comprehends an isolated nucleic acid molecule encoding human protein 608 or a functional portion thereof. The invention further comprehends an isolated nucleic acid molecule encoding human protein 405 or a functional portion thereof. And, the invention comprehends the isolated nucleic acid molecule encoding human protein 274 or a functional portion thereof. In particular embodiments, the invention provides the isolated nucleic acid molecules identified herein by sequence numbers, as well as functional portions thereof.

- 25 The invention further encompasses a vector comprising an inventive isolated nucleic acid molecule, a composition comprising such a vector, a probe or primer which specifically hybridizes to such an isolated nucleic acid molecule, and an expression product of such an isolated nucleic acid molecule.

- 30 The invention still further provides an isolated polypeptide herein identified as protein 608 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

The invention also provides an isolated polypeptide herein identified as protein 405 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

And, the invention provides an isolated polypeptide herein identified as protein 274 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

The invention comprehends an isolated polypeptide which is human protein 608 or a functional portion thereof, as well as an isolated polypeptide which is human protein 405 or a functional portion thereof, and an isolated polypeptide which is human protein 274 or a functional portion thereof. The invention further comprehends polypeptides identified by sequence identification numbers, as well as polypeptides from expression of nucleic acid molecules identified by sequence identification numbers; and, functional portions thereof. Further still, the invention comprehends compositions comprising an inventive polypeptide or portion thereof. Even further, the invention envisions an antibody elicited by an inventive polypeptide or a functional portion thereof, as well as a functional portion of such an antibody; and, compositions comprising such an antibody or portion thereof.

The invention further encompasses methods for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering an inventive polypeptide or portion thereof; and accordingly, the invention comprehends uses of polypeptides in preparing a medicament or therapy for such prevention, treatment or control.

The invention even further encompasses a method for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering an inventive vector or inventive nucleic acid molecules; and accordingly, the invention comprehends uses of such vectors or nucleic acid molecules in preparing a medicament or therapy for such prevention, treatment or control.

The invention also comprehends a method for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a

lack thereof, comprising administering a composition comprising a gene or functional portion thereof identified in inventive methods or an inventive model or an expression product thereof or an antibody or portion thereof elicited by such an expression product or portion thereof; and, the invention thus further comprehends uses of such
5 genes, expression products, antibodies, portions thereof, in the preparation of a medicament or therapy for such control, prevention or treatment.

The invention yet further provides methods for preparing a polypeptide comprising expressing the polypeptide from inventive vectors or from inventive genes or genes identified in inventive methods or models, or portions of such genes.

10 Further still, the invention envisions advancing research in or studies of bone development and/or osteoporosis comprising the inventive methods, materials/products, and/or models.

The invention comprehends genes differentially expressed under the influence of (a) mechanical force applied to a calvaria primary cell culture and (b) treatment of
15 PGE2 applied to the same culture. In addition the invention involves the effect of calcium depletion. The genes that are differentially expressed are thus demonstrated to be involved in the processes that lead to osteoporosis or other mechanical stress or lack thereof related conditions.

Certain genes identified by the methods herein respond to estrogen. From the
20 methods disclosed herein one can identify compounds to which genes identified by herein methods respond. Thus, the invention comprehends a method for affecting a gene identified by any one of the herein methods comprising contacting cells containing the gene with a compound to which the gene responds; e.g., administering the compound as a composition or formulation as herein described. Thus, for
25 instance, with respect to genes which respond to estrogen, the invention envisions a method for affecting (e.g., stimulating expression, inhibiting expression, and the like) the gene comprising contacting a cell containing the gene with estrogen or a derivative or precursor thereof, e.g., 17-Beta estradiol and the like.

It is noted that in this disclosure, the word "comprises" can have the meaning
30 attributed to it in U.S. Patent law; e.g., it can mean "includes".

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF FIGURES

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

Figure 1A shows an absorbance profile of a fractionation of cytoplasmic RNA on a sucrose density gradient wherein the absorbance (at 254nm) is plotted against the sedimentation rate of the cytoplasmic RNA;

Figure 1B shows a purified RNA electrophoresed on an agarose gel and stained with ethidium bromide illustrating the fractionation of RNA;

Figure 2 shows a representation of DNA chip hybridization results comparing probes of total RNA (Tot) to probes derived from nuclear RNA (STP);

Figure 2A shows a table of genes identified by inventive methods, and sequences therefor or sequences of ESTs thereof (SEQ ID NOS:);

Figure 3 shows DNA and amino acid sequences for inventive nucleic acid molecule 608 and the expression product therefrom with this Figure differing from other 608 sequences herein in that Figure 3 shows additional protein sequences towards the 5' end (compare Figure 3 from about position 1025 with other 608 sequence figures herein) (SEQ ID NOS:);

Figure 4 shows the results of a 5' fragment probe of inventive nucleic acid molecule 608 on target mRNA in normal and mechanically stressed cells;

Figure 5 shows DNA and amino acid sequences for inventive nucleic acid molecule 608 and the expression product therefrom (SEQ ID NOS:);

Figure 6 shows Clustal X (1.64b) Multiple Sequence Alignment with respect to inventive nucleic acid molecule 608 and probes therefor (SEQ ID NOS.);

Figures 7 shows the results of a probe of human 405 on the target total RNA of human k562;

Figure 8 shows the results of a probe of human 405 on the target rat cmf RNA;

Figures 9 and 10 show the DNA and amino acid sequences for inventive nucleic acid molecule 405 and the expression product therefrom (SEQ ID NOS:);

Figure 11 shows Clustal X (1.64b) Multiple Sequence Alignment with respect to inventive nucleic acid molecule 405 and probes therefor (SEQ ID NOS.);

Figure 12 shows the results of a probe of 8 KB of human 274 on the target rat bone, rat testes and human cell line NB4 total RNA sources;

Figure 13 shows the DNA and amino acid sequences for inventive nucleic acid molecule 274 and the expression product therefrom (SEQ ID NOS:);

5 Figure 14 shows the DNA and amino acid sequences for inventive nucleic acid molecule 274 and the expression product therefrom (SEQ ID NOS:).

(Markings on sequence figures, e.g., sequence figures such those for 608, such as dots and plus/minus signs may indicate repeats, such as IgG repeats, that may appear in many proteins; there are approximately 20 such IgG repeats in the 608 sequence

10 listing.)

DETAILED DESCRIPTION

As discussed, disclosed herein is a method for identifying genes whose expression is regulated at the RNA level in an organism.

More in particular, disclosed herein is a method of identifying genes whose
15 expression is regulated at least in part at the mRNA level by selectively stimulating an unknown target mRNA with a stress inducing element, the target mRNA being part of a larger sample. The organism may be any organism which provides suitable mRNA. The mRNA sample is derived from cellular compartments based on expression regulation and protein localization which are differentially analyzed to identify genes
20 which are translationally regulated by the stress inducing element. This method is designed for identifying and cloning genes which are responsive to specific cues. That is, the present method is designed for identifying and cloning genes which are either up- or down- regulated responsive to a specific pathology, stress, physiological condition, and so on, and in generally to any factor that can influence cells or
25 organisms to alter their gene expression.

This disclosure provides a novel approach to the identification and cloning of genes that are involved in fundamental cellular functions and which are regulated at any level in an organism. The basic underlying theory for this method relies on the knowledge that the regulation of gene expression can be controlled at different levels
30 (modes) and that each different regulation level(s) is manifested by some difference in the distribution of the specific mRNAs in the cell. In genes that are regulated by translation, the mRNA is stored in the cell in an inactive form and will not be found on polysomes. Following the appropriate external cue, the mRNA is incorporated into

the polysomes and translated, and the encoded protein quickly appears. By comparing mRNA populations that are "active" or "non-active" at a given time, genes that are regulated by a mechanism referred to as the "shift mechanism" can be identified.

5 Genes whose main regulatory level is the active transport of mRNA from the nucleus to the cytoplasm are stored in the nucleus and at the appropriate cue the mRNA is transported to the cytoplasm. Comparison of mRNA isolated from the nucleus and cytoplasm before and after the cue can lead to the discovery of genes controlled in this way. The comparison of mRNA derived from the nucleus also
10 allows direct analysis of the transcription activity of many genes. For most transcriptionally activated genes a basal level of mRNA exists in the cell even when the basal transcription activity is low. Thus, increased transcription (up to five-fold) is often obscured when total cellular RNA is used for differential analysis of gene expression. The use of nuclear RNA allows direct measurement of transcription
15 activity of many genes, since the basal mRNA is found in the cytoplasm. The result is a major increase in sensitivity for the detection of differential expression.

In the case of mRNA stability regulation, it is expected that such mRNA would be similarly transcribed before and after cue administration, resulting in a similar abundance in nuclear mRNA pools. However, if the mRNA is stabilized
20 following the cue, its abundance in the cytoplasm would become higher. In the case of mRNA transport regulation, such mRNA is expected to exist at a high level in the nucleus and a low level in the cytoplasm prior to the cue, which situation would be reversed after administration of the cue. It is thus easy to differentiate between the two regulatory modes.

25 The method of the invention includes the identification of genes regulated at the translational level; genes regulated at the transcription level; genes regulated by RNA stability; genes regulated by mRNA transport rate between the nucleus and the cytoplasm; and genes regulated by differential splicing. That is, genes whose expression is at least partly controlled or regulated at the mRNA level can be
30 identified.

The method will identify genes encoding secreted and membrane proteins; genes encoding for nuclear proteins; genes encoding for mitochondrial proteins; and

genes encoding for cytoskeletal proteins. In addition, any other gene whose expression can be controlled at the mRNA level can be identified by this method.

As used herein, RNA refers to RNA isolated from cell cultures, cultured tissues or cells or tissues isolated from organisms which are stimulated, differentiated,
5 exposed to a chemical compound, are infected with a pathogen or otherwise stimulated. As used herein, translation is defined as the synthesis of protein on an mRNA template.

As used herein, stimulation of translation, transcription, stability or transportation of unknown target mRNA or stimulating element, includes chemically,
10 pathogenically, physically, or otherwise inducing or repressing an mRNA population from genes which can be derived from native tissues and/or cells under pathological and/or stress conditions. In other words, stimulating the expression of a gene's mRNA with a stress inducing element or "stressor" can include the application of an external cue, stimulus, or stimuli which stimulates or initiates translation of a mRNA
15 stored as untranslated mRNA in the cells from the sample. The stressor may cause an increase in stability of certain mRNAs, or induce the transport of specific mRNAs from the nucleus to the cytoplasm. The stressor may also induce gene transcription. In addition to stimulating translation of mRNA from genes in native cells/tissues, stimulation can include induction and/or repression of genes under pathological
20 and/or stress conditions. The present method utilizes a stimulus or stressor to identify unknown target genes which are regulated at the various possible levels by the stress inducing element or stressor.

The method synergistically integrates methodologies which were not previously used together.

25 One methodology comprises the division of cellular mRNA into separate pools of mRNA derived from polysomes, nucleus, cytoplasm or spliceosomes.

Another methodology comprises the simultaneous comparison of the relative abundance of the mRNA species found in the separate pools by a method of differential analysis such as differential display, representational difference analysis
30 (RDA), gene expression microarray (GEM), suppressive subtraction hybridization (SSH) (Diatchenko et al., 1996), and oligonucleotide chip techniques such as the chip technology exemplified by United States Patent No. 5,545,531 to Rava et al. assigned

to Affymax Technologies N.V. and direct sequencing exemplified by WO 96/17957 patent application to Hyseq, Inc.

Briefly, subtractive hybridization is defined as subtraction of mRNA by hybridization in solution. RNAs that are common to the two pools form a duplex that can be removed, enriching for RNAs that are unique or more abundant in one pool. Differential Display is defined as reverse transcription of mRNA into cDNA and PCR amplification with degenerated primers. Comparison of the amounts amplification products (by electrophoresis) from two pools indicate transcript abundance. RDA, GEM, SSH, SAGE are described herein above.

10 The specific cells/tissues which are to be analyzed in order to identify translationally regulated genes, can include any suitable cells and/or tissues. Any cell type or tissue can be used, whether an established cell line or culture or whether directly isolated from an exposed organism.

15 The cells/tissues to be analyzed under the present method are selectively stimulated or "stressed" utilizing a physiological, chemical, environmental and/or pathological stress inducing element or stressor, in order to stimulate the translation of mRNA within the sample tissue and identify genes whose expression is regulated at least in part at the mRNA level. Stimulation can cause up or down regulation. Following stimulation, RNA is isolated or extracted from the cells/tissues. The isolation of the RNA can be performed utilizing techniques which are well known to those skilled in the art and are described, for example, in "Molecular Cloning; A Laboratory Manual" (Cold Springs Harbor Laboratory Press, Cold Spring Harbor, New York, 1989). Other methods for the isolation and extraction of RNA from cells/tissue can be used and will be known to those of ordinary skill in the art. (Mach et al., 1986, Jefferies et al., 1994). However, many variations of these methodologies have been published. The methods described herein were carefully selected after many trials.

20 The mRNAs which are actively engaged in translation and those which remain untranslated can be separated utilizing a procedure such as fractionation on a sucrose density gradient, high performance gel filtration chromatography, or polyacrylamide gel matrix separation (Ogishima et al., 1984, Menaker et al., 1974, Hirama et al., 1986, Mechler, 1987, and Bharucha and Murthy, 1992), since mRNAs that are being translated are loaded with ribosomes and, therefore, will migrate differently on a

density gradient than ribosome-free untranslated mRNAs. By comparing mRNA populations that are active or non-active in translation at a given time, genes that are regulated by the "shift mechanism" can be identified.

Polysomal fractionation and specific analysis can be facilitated by treatment of target cell/tissue with drugs that will specifically inhibit or modulate transcription or translation. Examples of such drugs are actinomycin D and cyclohexamide, respectively.

The fractionation can be completed to create polysomal subdivisions. The subdivisions can be made to discriminate between total polyribosomes or membrane bound ribosomes by methods known in the art (Mechler, 1987). Further, the mRNA sample can additionally be fractionated into one or more of at least the following subsegments or fractions: cytoplasmatic, nuclear, polyribosomal, sub polyribosomal, microsomal or rough endoplasmic reticulum, mitochondrial and spliceosome associated mRNA by methods known in the art.

More specifically, nuclear fractions can be obtained using the method set forth in the article entitled Abundant Nuclear Ribonucleoprotein Form of CAD RNA (Sperling, 1984) as set forth in the Examples, thus allowing nuclear RNA to be utilized for a method of identifying genes which are regulated or responsive to stress conditions. Further, antisense RNA can be utilized as a method for identifying genes which are responsive to specific pathology or stress conditions. Antisense RNA can be isolated using the methods described by Dimitrijevic, whose abstract details the methods utilized for obtaining and isolating antisense RNA from a sample. Additionally, microsomal fractions may be obtained using the methods of the present invention as set forth in the Experimental Section which are modifications of the methods disclosed by Walter and Blobel in 1983.

Following isolation and division of the total mRNA population into separate expression regulation and protein localization pools of mRNA, the relative abundance of the many mRNA species found in these pools are simultaneously compared using a differential analysis technique such as differential display, oligonucleotide chips, representational difference analysis (RDA), GEM-Gene Expression Microarrays (Schena et al., 1995, Aiello et al., 1994, Shen et al., 1995, Bauer et al., 1993, Liang and Pardee, 1992, Liang and Pardee, 1995, Liang et al., 1993, Braun et al., 1995, Hubank and Schatz, 1994) and suppressive subtraction hybridization (SSH). The

RNA isolated from the fractions can be further purified into mRNA without the ribosomal RNA by poly A selection. It should be noted that multiple pools can be analyzed utilizing this method. That is, different cell aliquots subjected to different stressors can be compared with each other as well as with the reference sample.

5 Labeled nucleic acid probes (in a cDNA, PCR product or rRNA transcribed from the cDNA) made from RNA derived from polysomal, non-polysomal, mRNPs, nuclear, cytoplasmic, or spliceosome fractions can be used as probes, to identify clones of cDNA, genomic clones, and mRNA species that are fixed onto a solid matrix-like microarrays such as (GEM), that shown in United States Patent Number
10 5,545,531 to Rava et al. and WO96/17957 to Hyseq, Inc., and membranes of any kind where clones can be either blotted after electrophoresis or directly loaded (dot blot) onto the membrane. The label can be radioactive, fluorescent, or incorporating a modified base such as digoxigenin and biotin.

 Comparison between the fractions derived from the polysomal or
15 polyribosomal fraction or other fractions to the total unfractionated material is essential to discriminate between differentials in expression levels that are the result of transcription modulation from those that result from modulation of translation per se. The polysomal fractions or groups can include membrane bound polysomes, loose or tight polysomes, or free unbound polysome groups.

20 The importance of utilizing the polysomal sub-population in order to identify differentially (translationally) expressed genes is shown in Example 1 where a number of genes were not detected as translationally expressed under heat shock inducement when total mRNA was used as the detection probe but, however, when polysomal mRNA was used as a probe, a number of genes were identified as
25 differentially expressed. As shown in Example 1, a number of genes under heat shock inducement with total mRNA derived probe were detected when probed with polysomal mRNA fractions. Heat shock, being a model for acute diseases such as ischemic diseases, reveal the importance of the polysomal probe. Cells store critical mRNAs in an inactive form so that in an acute situation they can be quickly loaded
30 onto polysomes (without the need to wait for their production by transcription) and translated to produce the proteins the cells require for their survival under stress.

 The present method for identifying translationally regulated genes is not limited by the source of the mRNA pools. Therefore, the present method can be

utilized to clone genes from native cells/tissue under pathological and/or stress conditions that are regulated by the "shift mechanism," as well as genes that are induced/repressed under pathological and/or stress conditions. Pathologies can include disease states including those diseases caused by pathogens and trauma.

5 Stress conditions can also include disease states, physical and psychological trauma, and environmental stresses. Following analysis by the selected method of differential analysis, the genes which have been identified as being regulated by translation can be cloned by any suitable cloning methodologies known to those skilled in the art. (Lisitsyn and Wigler, 1993).

10 Differential comparisons can be made of all possible permutations of polysomal vs. non-polysomal RNA where the definition of the fraction type is done, for example, by absorbance profile at 254nm, density of the sucrose gradient as shown in Figure 1A (or another size standard if high pressure liquid chromatography or gel systems are used) and types of RNA that are stained with ethidium bromide
15 after electrophoresis of the fractions on agarous gels are completed, as shown in Figure 1B. In Figure 1A, the polysomal fractions are those that have mRNA with more than two ribosomes loaded. The materials and methods for this comparison are set forth below in the experimental section.

Differential comparisons can also include polysomal vs. non-polysomal
20 fractions in each condition. By "condition" it is meant that cells from the same source, such as a cell line, a primary cell, or a tissue that undergoes different treatment or has been modified to have different features or to express different sets of genes. For example, this can be accomplished by differentiation, transformation, application of the stress such as oxygen deprivation, chemical treatment, or radiation.

25 Permutations can include, for example:

1. polysomal fractions between conditions individually (migrating in the same density) or in a pool;

2. non-polysomal fractions between conditions individually (migrating in the same density) or in a pool;

30 3. non-polysomal to polysomal between conditions and within each condition individually (migrating in the same density) or in a pool; and

4. each of the fractions being polysomal and non-polysomal individually (migrating in the same density) or in a pool that can be compared to total RNA that is unfractionated.

The method described above for the identification of genes regulated on the translational level has a number of applications. A particular application for this method is its use for the detection of changes in the pattern of mRNA expression in cells/tissue associated with any physiological or pathological change. By comparing the translated versus untranslated mRNAs, the effect of the physiological or pathological cue or stress on the change of the pattern of mRNA expression in the cell/tissue can be observed and/or detected. This method can be used to study the effects of a number of cues, stimuli, or stressors to ascertain their effect or contribution to various physiological and pathological activities of the cell/tissue. In particular, the present method can be used to analyze the results of the administrations of pharmaceuticals (drugs) or other chemicals to an individual by comparing the mRNA pattern of a tissue before and after the administration of the drug or chemical. This analysis allows for the identification of drugs, chemicals, or other stimuli which affect cells/tissue at the level of translational regulation. Utilizing this method, it is possible to ascertain if particular mRNA species are involved in particular physiological or disease states and, in particular, to ascertain the specific cells/tissue wherein the external stimulus, i.e., a drug, affects a gene which is regulated at the translational level.

The identification of a subgroup of genes regulated on the translational level involved a method for identifying gene sequences coding for internal ribosome entry sites (IRES), including the general steps of inhibiting 5' cap-dependant mRNA translation in a cell, collecting a pool of mRNA from the cells, and differentially analyzing the pool of mRNA to identify genes with sequences coding for internal ribosome entry sites. The inhibiting step can be further defined as selecting for non-5'-cap dependent mRNA translation or by incorporating a gene, such as a gene coding for a protease such as poliovirus 2A protease. The method can include the step of controlling the expression of the gene. The analyzing step can be further defined as differential display analysis, or as representational difference analysis, or as performing a gene expression microarray analysis. The method can include the further step of cloning genes identified as being translationally regulated. The

analyzing step can distinguish between polysomal fractions that migrate in the same density individually or in a pool. The analyzing step can distinguish between nonpolysomal fractions individually or as a pool. The analyzing step can distinguish between stimulated polysomal and nonpolysomal fractions individually or in a pool.

- 5 And, the analyzing step can distinguish between each of the polysomal and nonpolysomal fractions individually or in a pool compared to an unfractionated total RNA pool.

Utilizing these methods, it is possible to ascertain if particular mRNA species are involved in particular physiological or disease states and, in particular, to ascertain
10 the specific cells/tissue wherein the external stimulus, e.g., a drug, affects a gene which is regulated at the translational level.

- Accordingly, in an aspect, the application also discloses a method for determining risk of developing a physiological or disease state based upon absence or decrease from normal cells of mRNA or protein from a gene shown to be down
15 regulated by the inventive or herein disclosed methods in a mammal comprising:
- (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
 - (b) determining the level or status of corresponding protein in cells of said mammal; and
 - 20 (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity
25 of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

In another aspect, the disclosure herein provides a method for determining risk of physiological or disease state based upon presence or increase from normal cells of
30 mRNA or protein from a gene shown to be upregulated by the inventive or herein disclosed methods in a mammal comprising:

- (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or

(b) determining the level or status of corresponding protein in cells of said mammal; and

- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells of mRNA or protein and thus risk.

The foregoing methods can be employed in inventive methods for testing a medicament for or a gene therapy approach to a physiological or disease state or other factors causing or contributing thereto or to symptoms thereof based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein of identified genes additionally comprising: (a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or increase from normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

Similarly, in still further aspects, the disclosure herein provides methods for treating, preventing or controlling a physiological or disease state comprising administering a medicament or treatment therefor or for a cause thereof or a symptom thereof, including the foregoing detection methods. For instance, from the comparing one determines an absence or decrease from normal cells or presence or increase from normal cells of particular mRNA or protein and thus risk and administers a the medicament or treatment.

The methods can additionally comprise using the steps in conjunction with another test method akin to those described above, e.g., having a same or similar preamble recitation and comprising:

- (d) determining the level or status of a second gene mRNA in cells of said mammal; and/or
- (e) determining the level or status of protein expressed by a second gene product in cells of said mammal; and

- (f) comparing said level or status of that mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein.

The absence or decrease or presence or increase may be correlated to risk.

- Thus, the second gene can be identified by methods of the invention or as disclosed herein. Or alternatively or additionally, the second gene and/or the additional steps can be determined in accordance with other methods, e.g., other methods for determining the risk of the physiological or disease state or a condition or factor associated therewith. Thus, such methods can be used in conjunction with methods herein to advance or improve diagnostic or detection methodologies.

- In the methods, steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vivo* and/or steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vitro*.

The determination in step (a) and optionally in step (d) can be effected by employing

- (i) a nucleic acid sequence corresponding to at least a part of the gene encoding at least part of the protein and optionally a second nucleic acid sequence corresponding to at least a part of the second gene encoding at least part of the second protein;
- (ii) a nucleic acid sequence complementary to the nucleic acid sequence(s) of (i);
- or
- (iii) a primer or a primer pair hybridizing to the nucleic acid sequence(s) of (i) or (ii).

- The determination in step (b) and optionally of step (e) can be effected by employing an antibody or a fragment thereof that specifically binds to the protein and optionally by employing a second antibody or a fragment thereof which specifically binds to the second protein.

Cells may be considered "normal" in the methods by having an absence of the physiological or disease being tested for; or by any other standard definition recognized in the art.

The medicament or treatment can be any conventional medicament or treatment for the physiological or disease. Alternatively or additionally, the medicament or treatment can be the particular protein of the gene detected in the inventive methods or a functional portion thereof, or that which inhibits that protein, e.g., binds to it. Similarly, additionally or alternatively, the medicament or treatment can be a vector which expresses the protein of the gene detected in the inventive methods or a functional portion thereof or that which inhibits expression of that gene; again, for instance, that which can bind to it and/or otherwise prevents its transcription or translation. The selection of administering a protein or that which expresses it, or of administering that which inhibits the protein or the gene expression, can be done without undue experimentation, e.g., based on down regulation or up regulation as determined by inventive methods (e.g., in the osteoporosis model).

In an aspect of the invention, the stimulus in inventive methods is mechanical stress or a lack thereof, e.g., with respect to bone cells which retain their characteristic thereof in cultures.

In a further aspect, the invention provides an application of inventive methods with respect to osteoporosis, a major health problem; and provides inventive products and uses therefor. As discussed, osteoporosis or porous bone, is a progressive and chronic disease characterized by low bone mass and structural deterioration of bone tissue, with bone loss being possibly without symptoms, leading to bone fragility and an increased susceptibility to fractures of the hip, spine, and wrist (diminishing bone strength).

Osteoporosis is histologically, biochemical and kinetically heterogeneous. Data points to causes such as: deficiency of estrogen and deficiency of calcium.

Calcium is an essential nutrient that is involved in most metabolic processes and the phosphate salts of which provide mechanical rigidity to the bones and teeth, where 99% of the body's calcium resides. The calcium in the skeleton has the additional role of acting as a reserve supply of calcium to meet the body's metabolic needs in states of calcium deficiency. Calcium deficiency is easily induced because of the obligatory losses of calcium via the bowel, kidneys, and skin. Calcium deficiency delays the consolidation of the skeleton, may cause mobilization of bones and has been shown in animals to lead to osteoporosis.

Further, bone is composed of a collagen-rich organic matrix impregnated with mineral, largely calcium and phosphate. Two major forms of bone exist, compact cortical bone forms the external envelopes of the skeleton and trabecular or medullary bone forms plates that traverse the internal cavities of the skeleton. The responses of these two forms to metabolic influences and their susceptibility to fracture differ. Bone undergoes continuous remodeling (turnover) throughout life. Osteoclasts are the cells in the skeleton that responsible for breaking down bones, osteoblasts on the other hand, are capable of forming new bones. Mechanical and electrical forces, hormones and local regulatory factors influence remodeling. Peak bone mass is mainly genetically determined, though dietary factors and physical activity can have positive effects. Peak bone mass is attained at the point when skeletal growth ceases, after which time bone loss starts. Bone mass declines throughout life due to an imbalance in this process.

It is noted that the World Health Organization (WHO Technical Report Series: 843, 1994) characterizes "normal", e.g., as to women, as bone mineral density (BMD) or bone mineral content (BMC) that is greater than or equal to 1 standard deviation (SD) below the young adult reference range; "low bone mass" as BMD or BMC 1-2.5 SD below the mean of young healthy adults, e.g., women; "osteoporosis" as BMD or BMC greater than 2.5 SD below the mean of young healthy adults, e.g., women; and "severe osteoporosis" as BMD or BMC greater than 2.5 SD below the mean of young healthy adults, e.g., women and the presence of one or more fragility fractures. From this information and the knowledge in the art, the skilled artisan can determine and employ "normal" cells, without any undue experimentation.

Osteoblasts are particularly sensitive to aging phenomena--more sensitive than are osteoclasts--so the negative bone balance increases with increasing age. Age-dependent bone loss is aggravated by reduced calcium absorption, a mutation in the collagen gene and polymorphism in TGF-beta and estrogen receptor proteins.

Cells bind to ECM (extracellular matrix) via specific cell surface receptors such as integrins. When engaging with ECM ligands, these receptors can activate signal transduction pathways within the cells and may act as mechanochemical transducers. Thus, interaction of cells with ECM can modulate gene expression. Among the genes that are, in part, controlled by cell-ECM interactions are those for

certain ECM components themselves. Bone cells, remodel their matrix and reorient bone trabeculae in response to mechanical strain.

Accordingly, the nature of the bone cell response can relate to the state of differentiation. Furthermore, evidence shows that prostaglandins are likely to play an important role in the physiologic and pathologic responses of bone tissue.

5 Prostaglandins can stimulate and inhibit bone resorption and formation. Prostaglandins mediate bone loss due to immobilization, but prostaglandin E2 (PGE2) stimulates bone formation *in vivo*. Prostaglandin production by bone cells is highly regulated by mechanical forces, cytokines, growth factors and systemic hormones.

10 Mechanical stimulation applied to cultured bone cells results in increased production of several prostaglandins including PGE2, prostaglandin I2 (PGI2), and prostaglandin F2a. Addition of indomethacin, which blocks endogenous prostaglandin production, neutralizes the effect of mechanical stress treatment.

Cells isolated from calvaria bone maintain their osteoblastic phenotype in culture. Genetics factors play an important role in the pathogenesis of osteoporosis. It is suggested that up to 85%-90% of the variance in bone mineral density is genetically determined. Thus, calvaria bone cells were used in methods of the invention. Genes differentially expressed under the influence of (a) mechanical force applied to a calvaria primary cell culture and (b) treatment of PGE2 applied to the same culture. In addition, the effect of calcium depletion is also shown. The genes that result

15 differentially expressed are thus demonstrated to be involved in the processes that lead to osteoporosis, and ergo osteoporosis.

20

It is well accepted that the main process that is characteristic of osteoporosis – enhanced bone resorption – takes place not only in conditions of low estrogen production (menopause women), but also in some other conditions, like treatment with glucocorticoids or bone immobilization. Therefore, it was reasoned that application of mechanical force is stimulatory for bone formation. To model this process, as discussed in the Examples, e.g., Example 2, primary rat calvaria cells grown on elastic membranes and stretched together with this membrane for 20

25 minutes. Genes expression patterns were compared before and after the application of mechanical force. Particular genes were found to be differentially regulated and/or differentially expressed following mechanical stimulation, validating the osteoporosis model; and, showing that the inventive methods can be used to identify genes,

30

expression products therefrom, probes/primers for such genes, as well as uses for such genes, expression products, probes/primers, *inter alia*.

In an aspect the invention provides a gene identification process. Steps involved in the gene identification process comprise one or more or all of the following: Preparation of probes from the model system (mechanical force, calvaria primary culture); analysis of DNA chip hybridization; sequencing of clones showing differential expression; and full-length cloning of clones of interest (cloning can be by a variety of known methodologies).

In yet another aspect the invention provides an osteoporosis model or a model for other conditions caused by mechanical stress or force, e.g., bone mass formation, comprising rat calvaria cells or another cell which retains osteoblast or osteoclast nature in cell cultures, being subjected to mechanical or other bone growth/formation inducing stress or stimuli or bone loss inducing stress or stimuli.

With respect to mechanical stress and osteoporosis, it is well documented that exercise has very beneficial effects on bone mass. The effect of the zero of gravity on astronauts and their need to do a lot of exercise is also believed well known.

However, as far as the inventors know, efforts to isolate genes involved in the biological interpretation of mechanical stress signals into increase in bone mass have not heretofore been done. And, mention is made of: Binderman I, Duksin D, Harell A, Katzir E, Sachs L. Formation of bone tissue in culture from isolated bone cells. J Cell Biol 1974 May;61(2):427-39; Harell A, Dekel S, Binderman I. Biochemical effect of mechanical stress on cultured bone cells. Calcif Tissue Res 1977 May;22 Suppl:202-7; Somjen D, Binderman I, Berger E, Harell A. Bone remodelling induced by physical stress is prostaglandin E2 mediated. Biochim Biophys Acta 1980 Jan 3;627(1):91-100; Shimshoni Z, Binderman I, Fine N, Somjen D. Mechanical and hormonal stimulation of cell cultures derived from young rat mandible condyle. Arch Oral Biol 1984;29(10):827-31; Binderman I, Shimshoni Z, Somjen D. Biochemical pathways involved in the translation of physical stimulus into biological message. Calcif Tissue Int 1984;36 Suppl 1:S82-5; Binderman I, Zor U, Kaye AM, Shimshoni Z, Harell A, Somjen D. The transduction of mechanical force into biochemical events in bone cells may involve activation of phospholipase A2. Calcif Tissue Int 1988 Apr;42(4):261-6; Binderman I, Berger E, Fine N, Shimshoni Z, Harell A, Somjen D. Calvaria derived

osteogenic cells: phenotypic expression in culture. Connect Tissue Res 1989;20(1-4):41-7.

The inventive osteoporosis model or model for other conditions caused by mechanical stress or force or lack thereof, encompasses inventive methods and products of inventive methods employed under conditions of little or no gravity, e.g., the results of performing inventive methods, such as those exemplified or analogous to those exemplified, for instance without applying mechanical stress and under conditions of little or no gravity such as on a space vehicle such as a Space Shuttle or a space station as is being constructed.

10 The invention is still further aspects provides CMF274, expression products therefrom, probes/primers therefor, and uses for such gene, expression products and primers/probes, as well as of functional portions of the gene or of the expression product.

15 The invention is still further aspects provides CMF405, expression products therefrom, probes/primers therefor, and uses for such gene, expression products and primers/probes, as well as of functional portions of the gene or of the expression product.

20 The invention is still further aspects provides CMF608, expression products therefrom, probes/primers therefor, and uses for such gene, expression products and primers/probes, as well as of functional portions of the gene or of the expression product.

Species of origin of the sequences: all initial sequences (short fragments of ~500bp) were rat. For 405 a homologue in the form of a partially characterized mRNA was found but there is no published information on its expression in bones. 25 The study in rats was done to prove its function and possible uses in humans (directly or indirectly) for therapeutics. Once the rat sequence is known the isolation of the human homologues can be within the ambit of the skilled artisan; and thus, this disclosure is intended to cover the human homologues as well because these homologues fall within a degree of homology included within the present invention.

30 More in particular, respect to the herein mentioned nucleic acid molecules and polypeptides therefrom, e.g., the aforementioned nucleic acid molecules (608, 405, 274) and polypeptides expressed from them, the invention further comprehends isolated and/or purified nucleic acid molecules and isolated and/or purified

polypeptides having at least about 70%, preferably at least about 75% or about 77% identity or homology ("substantially homologous or identical"), advantageously at least about 80% or about 83%, such as at least about 85% or about 87% homolgy or identity ("significantly homologous or identical"), for instance at least about 90% or
5 about 93% identity or homology ("highly homologous or identical"), more advantageously at least about 95%, e.g., at least about 97%, about 98%, about 99% or even about 100% identity or homology ("very highly homologous or identical" to "identical"; or from about 84-100% identity considered "highly conserved"). The invention also comprehends that these nucleic acid molecules and polypeptides can be
10 used in the same fashion as the herein or aforementioned nucleic acid molecules and polypeptides.

Nucleotide sequence homology can be determined using the "Align" program of Myers and Miller, ("Optimal Alignments in Linear Space", CABIOS 4, 11-17, 1988, incorporated herein by reference) and available at NCBI. Alternatively or
15 additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the
20 sequences. Hence, the DNA sequence AGTCAGTC will have a sequence similarity of 75% with the sequence AATCAATC ($N_{ref} = 8$; $N_{dif} = 2$).

Alternatively or additionally, "homology" or "identity" with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two
25 sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman, 1983 PNAS USA 80:726, incorporated herein by reference), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be
30 conveniently performed using commercially available programs (e.g., Intelligenetics™ Suite, Intelligenetics Inc. CA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T)

in the DNA sequence is considered equal to uracil (U) in the RNA sequence (*see also* alignment used in Figures).

RNA sequences within the scope of the invention can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

Additionally or alternatively, amino acid sequence similarity or identity or homology can be determined using the BlastP program (Altschul *et al.*, Nucl. Acids Res. 25, 3389-3402, incorporated herein by reference) and available at NCBI. The following references (each incorporated herein by reference) provide algorithms for comparing the relative identity or homology of amino acid residues of two proteins, and additionally or alternatively with respect to the foregoing, the teachings in these references can be used for determining percent homology or identity: Needleman SB and Wunsch CD, "A general method applicable to the search for similarities in the amino acid sequences of two proteins," J. Mol. Biol. 48:444-453 (1970); Smith TF and Waterman MS, "Comparison of Bio-sequences," Advances in Applied Mathematics 2:482-489 (1981); Smith TF, Waterman MS and Sadler JR, "Statistical characterization of nucleic acid sequence functional domains," Nucleic Acids Res. 11:2205-2220 (1983); Feng DF and Doolittle RF, "Progressive sequence alignment as a prerequisite to correct phylogenetic trees," J. of Molec. Evol., 25:351-360 (1987); Higgins DG and Sharp PM, "Fast and sensitive multiple sequence alignment on a microcomputer," CABIOS, 5: 151-153 (1989); Thompson JD, Higgins DG and Gibson TJ, "ClusterW: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, positions-specific gap penalties and weight matrix choice," Nucleic Acid Res., 22:4673-480 (1994); and, Devereux J, Haeberlie P and Smithies O, "A comprehensive set of sequence analysis program for the VAX," Nucl. Acids Res., 12: 387-395 (1984).

In this fashion, by comprehending nucleic acid molecules and polypeptides having such homology to the particular sequences disclosed, it is envisioned that the invention encompasses human and other homologues to the disclosed sequences, within the herein terms. Identification and/or isolation of corresponding human sequences can be any suitable method, for instance, by analysis of hybridization of herein defined genes (such as genes identified herein and/or identified by inventive methods herein) or suitable portions thereof, e.g., primers/probes derived from herein

defined genes; for instance, in PCR amplification of portions of the human genome by such primers/probes and/or labeled hybridization analysis of herein defined genes or portions thereof to portions of the human genome (*see also* discussions *infra*, e.g., concerning PCR, hybridization, *inter alia*).

5 Furthermore, by comprehending proteins having homology to the gene products of genes identified herein (e.g., 405, 608, 274) as well as of genes identified by the methods disclosed herein, the invention comprehends proteins which are "functional" proteins derived from gene products identified herein, as well as from gene products of genes identified by the methods disclosed herein; e.g., truncated
10 forms of proteins identified herein or expressed by genes identified by the methods disclosed herein.

As to uses, the inventive genes and expression products as well as genes identified by the herein disclosed methods and expression products thereof (including "functional" variations of such expression products, and ergo truncated portions of
15 herein defined genes such as portions of herein defined genes which encode a functional portion of an expression product) are useful in treating, preventing or controlling or diagnosing or observing or studying osteoporosis or processes thereof or mechanical stress conditions or absence or reduced mechanical stress conditions. They may aid in bone density. They may be useful for diagnostic purposes. They
20 may be used for determining predisposition to high or low bone density or for determining gene association or other factors associated with high bone mass or low bone mass.

For instance, 608 expression causes cells to differentiate into osteoblasts and chondrocytes. The expression product of 608, or if cells or vectors expressing 608
25 may cause cells to selectively differentiate and thereby increase or alter bone density. Detecting levels of 608 mRNA or expression and comparing it to "normal" non-osteopathic levels may allow one to detect who may be at risk for osteoporosis or lower levels of osteoblasts and chondrocytes.

405 expression impacts upon bone density by being characteristic for
30 osteogenic and chondrogenic cells in their differentiation preceeding matrix calcification. The expression product of 405 or cells or vectors expressing it may cause cells to differentiate into osteogenic and chondrogenic cells and thereby increase matrix calcification and bone density. One may detect a risk of low bone

density or low matrix calcification or osteoporosis by determining levels of expression of 405 or of mRNA and compare it to "normal" levels.

274 is implicated in lymphoid precursors in bone marrow. Under expression may lead to less lymphoid cells and bones that are more susceptible to environmental factors or other than genetic factors of osteoporosis, e.g., cancer causes of osteoporosis. One may detect a risk of predisposition of bones towards susceptibility to environmental factors, or less lymphoid cells, or osteoporosis by determining levels of expression of 405 or of mRNA and comparing it to "normal" levels.

Further, genes which were upregulated and identified by the method of the present invention are of interest. That which may inhibit these genes and/or the expression products therefrom or portions thereof, e.g., antibodies or functional portions thereof or other compounds which bind thereto, may be useful in preventing, controlling or treating osteoporosis or factors leading thereto or causing osteoporosis or other conditions involving mechanical stress or a lack thereof, and the genes may be targets for anti-osteoporosis treatment or therapy, as well as for study of osteoporosis or factors leading thereto or causes thereof, e.g., determining predisposition to high or low bone density or for determining gene association or other factors associated with high bone mass or low bone mass.

Among these, three identified upregulated RGD-containing proteins, ADAMTS-1 and complement 3 itself (potential prevention of osteoclast attraction) and two proteins of the SARP family (secreted apoptosis related proteins) as potential modifiers of programmed cell death in bone formation were identified.

Similarly, genes which were downregulated and identified by the method of the present invention are interesting. These genes and/or the expression products therefrom and/or a functional portion thereof may be useful in preventing, controlling or treating osteoporosis or factors leading thereto or causing osteoporosis, or other conditions involving mechanical stress or a lack thereof, and the genes may be targets for anti-osteoporosis treatment or therapy, as well as for study of osteoporosis or factors leading thereto or causes thereof, e.g., determining predisposition to high or low bone density or for determining gene association or other factors associated with high bone mass or low bone mass.

Accordingly, in an aspect, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or

contributing to osteoporosis or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells of mRNA or protein from a gene shown to be down regulated by the inventive methods in a mammal comprising:

- 5 (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
- (b) determining the level or status of corresponding protein in cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding
- 10 level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including
- 15 post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

In another aspect, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or other conditions involving mechanical stress or a lack thereof, based upon presence or increase from normal cells of mRNA or protein from a gene shown to be upregulated by the inventive methods in a mammal comprising:

- 20 (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
- (b) determining the level or status of corresponding protein in cells of said
- 25 mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a
- 30 mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells of mRNA or protein and thus risk.

As mentioned, 608 expression causes cells to differentiate into osteoblasts and chondrocytes. Thus, in a further aspect, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or lower levels of osteoblasts and chondrocytes or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells of mRNA or protein from 608 comprising:

- (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
- 10 (b) determining the level or status of corresponding protein in cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a
- 15 transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells of mRNA or protein and thus risk.

20 As discussed herein, 405 expression impacts upon bone density by being characteristic for osteogenic and chondrogenic cells in their differentiation preceeding matrix calcification. Accordingly, in a still further aspect, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of imbalance as to

25 osteogenic and chondrogenic cells or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells or presence or increase from normal cells, e.g., absence or decrease from normal cells of mRNA or protein from 405 in a mammal comprising:

- (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
- 30 (b) determining the level or status of corresponding protein in cells of said mammal; and

- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

Likewise, as discussed herein, 274 is implicated in lymphoid precursors in bone marrow.

Therefore, in yet another aspect, the invention provides a method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of being susceptible to environmental factors or other than genetic factors of osteoporosis, e.g., cancer causes of osteoporosis or of predisposition of bones towards susceptibility to environmental factors, or less lymphoid cells, or osteoporosis, or other conditions involving mechanical stress or a lack thereof, based upon presence or increase from normal cells or absence or decrease from normal cells, e.g., absence or decrease from normal cells of mRNA or protein from 274 in a mammal comprising:

- (a) determining the level or status of mRNA in cells, e.g., bone cells (for instance, osteoblasts and/or osteoclasts) of said mammal; and/or
- (b) determining the level or status of corresponding protein in cells of said mammal; and
- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

The foregoing methods, in still further aspects of the invention, can be employed in inventive methods for testing a medicament for or a gene therapy

approach to osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein of identified genes additionally comprising:

- 5 (a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or increase from normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

Similarly, in still further aspects, the invention provides methods for treating,
10 preventing or controlling osteoporosis or other conditions involving mechanical stress or a lack thereof, comprising administering a medicament or treatment for osteoporosis or a cause thereof or a symptom thereof, including the foregoing detection methods. For instance, from the comparing one determines an absence or decrease from normal cells or presence or increase from normal cells of particular
15 mRNA or protein and thus risk and administers a the medicament or treatment.

The cells in the inventive methods can be *in vitro* or *in vivo* or from any suitable mammal, e.g., a human, a domesticated animal, for instance a companion animal or livestock, or a laboratory animal, such as a rat, mouse or the like; and, the cells can be from any stage of the mammal's development, such as embryonic, mature
20 or adult, immature or child, newborn, or elderly, and the like.

It is noted that as to CMF608, the inventors did not see any differences in its expression between normal and ovariectomized rats suggesting it may not necessarily *per se* be a marker for bone intensity. Similarly, for CMF405 there was no change in expression after ovariectomy. In addition, expression in a few non-bone tissues
25 suggests it may not necessarily *per se* be a marker.

Thus, inventive methods can additionally comprise using the steps in conjunction with another test method akin to those described herein, e.g., having a same or similar preamble recitation and comprising:

- (d) determining the level or status of a second gene mRNA in bone cells of said
30 mammal; and/or
(e) determining the level or status of protein expressed by a second gene product in bone cells of said mammal; and

- (f) comparing said level or status of that mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein.

The absence or decrease or presence or increase detected may be correlated to risk. Thus, the second gene can be identified by methods of the invention. Or, alternatively or additionally, the second gene and/or the additional steps can be determined in accordance with other methods, e.g., as in U.S. Patents Nos. 5,834,200 and/or 5,691,153.

Likewise, it is within the invention that the inventive genes or genes identified by inventive methods herein or portions thereof can be the subject of other or analogous methods, such as a method for determining predisposition to high or low bone density comprising detecting the under or over expression of the gene or abnormalities in a receptor for a gene product or polymorphysim; *see, e.g.*, U.S. Patent Nos. 5,834,200 and 5,691,153; for instance, the inventive genes or genes identified by inventive methods herein can be used in methods analogous to those of U.S. Patent Nos. 5,834,200 and 5,691,153.

In the inventive methods steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vivo* and/or steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vitro*.

The determination in step (a) and optionally in step (d) can be effected by employing

- (i) a nucleic acid sequence corresponding to at least a part of the gene encoding at least part of the protein and optionally a second nucleic acid sequence corresponding to at least a part of the second gene encoding at least part of the second protein;
- (ii) a nucleic acid sequence complementary to the nucleic acid sequence(s) of (i);
- or
- (iii) a primer or a primer pair hybridizing to the nucleic acid sequence(s) of (i) or (ii).

(Note also the discussion herein, e.g., *infra*, concerning primers/probes and PCR and hybridization.)

The determination in step (b) and optionally of step (e) can be effected by employing an antibody or a fragment thereof that specifically binds to the protein and
5 optionally by employing a second antibody or a fragment thereof which specifically binds to the second protein. (Note also the discussion herein, e.g., *infra*, concerning antibodies and methods for making and uses thereof.)

The medicament or treatment can be any conventional medicament or treatment for osteoporosis. Alternatively or additionally, the medicament or treatment
10 can be the particular protein of the gene detected in the inventive methods, or that which inhibits that protein, e.g., binds to it. Similarly, additionally or alternatively, the medicament or treatment can be a vector which expresses the protein of the gene detected in the inventive methods or that which inhibits expression of that gene; again, for instance, that which can bind to it and/or otherwise prevents its
15 transcription or translation. The selection of administering a protein or that which expresses it, or of administering that which inhibits the protein or the gene expression, can be done without undue experimentation, e.g., based on down regulation or up regulation as determined by inventive methods (e.g., in the osteoporosis model).

In the practice of the invention, one can employ general methods in molecular
20 biology: Standard molecular biology techniques known in the art and not specifically described are generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1989, 1992), and in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989).

25 To determine the absence or decrease from normal cells or presence or increase from normal cells of a nucleic acid molecule, or to amplify it, e.g., in using probes or primers described herein or derived from nucleic acid molecules disclosed herein, the polymerase chain reaction (PCR) may be used and is conveniently carried out generally as in *PCR Protocols: A Guide To Methods And Applications*, Academic
30 Press, San Diego, CA (1990). Reactions and manipulations involving other nucleic acid techniques, unless stated otherwise, are performed as generally described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, and methodology as set forth in United States patents 4,666,828;

4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference. *In-situ* (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al, 1996, Blood 87:3822.)

5 In PCR, as well as in hybridization, it is preferred that the primers (or probes) bind specifically to the gene of interest, e.g., an inventive gene disclosed herein such as 608, 405 or 274, or a gene identified by methods disclosed herein, or a corresponding human homolog being detected by primer(s) or probe(s) derived from a herein defined gene. One way to ensure this is to select primers from the gene
10 sequence that are not generally found in other known sequences.

The invention accordingly in yet a further aspect provides an isolated nucleic acid molecule, e.g., DNA comprising a sequence encoding a herein defined gene or encoding a herein defined a polypeptide (e.g., an expression product of a herein defined gene) comprising at least about 12 nucleotides in length, for instance, at least
15 about 15, about 18, about 21, about 24 or about 27 nucleotides in length, such as at least about 30, about 33, about 36, about 39 or about 42 nucleotides in length, for example, a nucleic acid molecule of at least about 12 nucleotides in length such as about 12 to about 30, about 12 to about 50 or about 12 to about 60, or about 12 to about 75 or about 12 to about 100 or more nucleotides in length. Nucleic acid
20 molecules of these lengths may be useful in hybridization; and, the invention further comprehends vectors or plasmids containing and/or expressing such a nucleic acid molecule, as well as uses of such nucleic acid molecules, e.g., for expression thereof either *in vitro* or *in vivo*, or for amplifying or detecting a herein defined gene or a homolog thereof, e.g., a human homolog, in a sample, for instance by a polymerase
25 chain reaction.

A probe or primer can be any stretch of at least 8, preferably at least 10, more preferably at least 12, 13, 14, or 15, such as at least 20, e.g., at least 23 or 25, for instance at least 27 or 30 nucleotides in a herein defined gene which are unique thereto. As to PCR or hybridization primers or probes and optimal lengths therefor,
30 reference is also made to Kajimura et al., GATA 7(4):71-79 (1990). The invention will thus be understood to provide oligonucleotides, such as , pairs of oligonucleotides, for use as primers for the *in vitro* amplification of DNA samples and fragments thereof, or for use in expressing a portion of DNA, either *in vitro* or *in vivo*.

The oligonucleotides preferably specifically hybridize to sequences flanking a nucleic acid to be amplified, wherein the oligonucleotides hybridize to different and opposite strands of the double-stranded DNA target. The oligonucleotides of the invention are preferably derived from the nucleic acid molecules, e.g., a herein defined gene, and teachings herein. As used in the practice of this invention, the term "derived from" is intended to encompass the development of such oligonucleotides from the nucleic acid molecules and herein defined gene(s) and teachings disclosed herein, from which a multiplicity of alternative and variant oligonucleotides can be prepared.

The term "specific hybridization" will be understood to mean that the nucleic acid probes of the invention are capable of stable, double-stranded hybridization to gene-derived DNA or RNA under conditions of high stringency, as the term "high stringency" would be understood by those with skill in the art (see, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Hames and Higgins, eds., 1985, Nucleic Acid Hybridization, IRL Press, Oxford, U.K.). Hybridization will be understood to be accomplished using well-established techniques, including but not limited to Southern blot hybridization, Northern blot hybridization, *in situ* hybridization and, most preferably, Southern hybridization to PCR-amplified DNA fragments.

The nucleic acid hybridization probe of the invention may be obtained by use of the polymerase chain reaction (PCR) procedure, using appropriate pairs of PCR oligonucleotide primers as provided herein or derived from the gene sequence(s) provided herein. See U.S. Pat. Nos. 4,683,195 to Mullis et al. and 4,683,202 to Mullis. The invention in a still further aspect provides oligonucleotides for *in vitro* amplification using any of a variety of amplification protocols known in the art. Preferably, the invention provides oligonucleotides for performing polymerase chain reaction (PCR). See U.S. Pat. Nos. 4,683,195 to Mullis et al. and 4,683,202 to Mullis.

The invention will thus be understood to provide oligonucleotides, specifically, pairs of oligonucleotides, for use as primers for the *in vitro* amplification of genes as disclosed herein, e.g., of DNA samples and fragments thereof. In the practice of this invention, the pairs of oligonucleotides herein provided will be understood to comprise two oligonucleotides, comprising from about 8 to about 30 nucleotide residues apiece, said oligonucleotides specifically hybridizing to sequences

flanking a nucleic acid to be amplified, wherein the oligonucleotides hybridize to different and opposite strands of the DNA target. The oligonucleotides of the invention are preferably derived from the nucleic acid primers discussed below or from the gene(s) disclosed herein. As used in the practice of this invention, the term "derived from" is intended to encompass the development of such oligonucleotides from the nucleic acid sequence of the gene(s) or the primers herein disclosed, from which a multiplicity of alternative and variant oligonucleotides can be prepared. In particular, the invention provides oligonucleotides having a sequence that is substantially complementary to the corresponding sequence of the nucleic acid hybridization probe. As used herein, the term "substantially corresponding to" is intended to encompass oligonucleotides comprising sequence additions, deletions and mismatches, wherein certain nucleotide residues of the oligonucleotide sequence are not optimally complementary (e.g., A-C or G-T) or are non-complementary (e.g., A-G or T-C) to the corresponding sequence of the nucleic acid hybridization probe, provided that such oligonucleotides retain the capacity to specifically amplify the gene(s).

Nucleic acids, e.g., 405, 608 or 274, and oligonucleotides therefrom, such as primers disclosed herein and derivable from the sequences of the present invention (e.g., portions of each disclosed gene which are about 8 to 30 or more nucleotides in length and bind with sufficient specificity to the gene are useful as diagnostic tools for detecting the existence of a osteoporosis or conditions or factors of osteoporosis. Such diagnostic or detection reagents comprise nucleic acid hybridization probes of the invention and encompass paired oligonucleotide PCR primers, as described above.

Methods provided by the invention include blot hybridization, *in situ* hybridization and *in vitro* amplification techniques for detecting osteoporosis or conditions or factors of osteoporosis in a sample such as a biological sample. Appropriate biological samples advantageously screened using the methods described herein include blood, serum, saliva and other body fluids, and other potential sources of infection.

In the detection methods of the invention, production of a specific DNA fragment produced by *in vitro* amplification of a template DNA sample is detected by agarose gel electrophoresis, ethidium bromide staining and ultraviolet transillumination of ethidium bromide stained gels, performed using conventional

techniques (Sambrook et al., *supra*), or detection by sequence detection systems using fluorogenic or other labeled probes that rely on automatic or automated detection instrumentation. In instances where a greater degree of specificity is required, hybridization of such agarose gels probed with a detectably-labeled nucleic acid hybridization probe of the invention is performed using standard techniques (Sambrook et al., *supra*). In each of these embodiments of the methods of the invention, a sufficient amount of a specific PCR-amplified DNA fragment is produced to be readily detected. For the purposes of this invention, the term "a sufficient amount of a specific PCR-amplified DNA fragment" is defined as that amount required to be detected, either by visualization of ethidium bromide-stained agarose gels or autoradiographic or other development of a blot hybridized with a detectably-labeled probe.

It will be understood that a sufficient quantity of a specific PCR amplified DNA fragment is prepared in PCR amplification reactions by performing a number of cycles required to produce said sufficient amount of the specific DNA fragment. The number of cycles in each PCR required to produce said sufficient amount of a specific DNA fragment will be understood to depend on the oligonucleotide primers, buffers, salts and other reaction components, the amount of template DNA and the PCR cycling times and temperatures. It will also be understood that the optimization of these parameters are within the skill of the worker of ordinary skill to achieve with no more than routine experimentation.

Detectably-labeled probes as provided by the invention are labeled with biotin, a radioisotope (including ^3H , ^{14}C , ^{35}S and ^{32}P), a fluorescent label (including fluorescein isothiocyanate), and an antigenic label. The detectable label is incorporated into the probe during synthetic preparation of the probe, whereby the probe is alternatively end-labeled or labeled by the incorporation of labeled nucleotides into the synthesized probe.

The invention also provides a PCR-based method for preparing a nucleic acid hybridization probe of the invention. In these embodiments, template DNA comprises a recombinant genetic construct of the invention. A detectably-labeled nucleic acid hybridization probe is prepared by performing PCR amplification using a pair of oligonucleotide primers specific for sequences flanking the position of the nucleic

acid insert. Detectable label is incorporated into the nucleic acid hybridization probe by direct end-labeling of PCR primers or incorporation of detectably-labeled nucleotide triphosphates into the probe nucleic acid.

PCR comprising the methods of the invention is performed in a reaction mixture comprising an amount, typically between <10 ng-200 ng template nucleic acid; 50-100 pmoles each oligonucleotide primer; 1-1.25 mM each deoxynucleotide triphosphate; a buffer solution appropriate for the polymerase used to catalyze the amplification reaction; and 0.5-2 Units of a polymerase, most preferably a thermostable polymerase (e.g., Taq polymerase or Tth polymerase).

The invention thus provides in further aspects diagnostic assays for the specific detection of osteoporosis or genes associated therewith. These diagnostic assays include nucleic acid hybridization assays, using the nucleic acids of the invention or specifically-hybridizing fragments thereof, for sensitive detection of fungal genomic DNA and/or RNA. Such assays include various blot assays, such as Southern blots, Northern blots, dot blots, slot blots and the like, as well as *in vitro* amplification assays, such as the polymerase chain reaction assay (PCR), reverse transcription-polymerase chain reaction assay (RT-PCR), ligase chain reaction assay (LCR), and others known to those skilled in the art. Specific restriction endonuclease digestion of diagnostic fragments detected using any of the methods of the invention, analogous to restriction fragment linked polymorphism assays (RFLP) are also within the scope of this invention.

These PCR techniques can be used in conjunction with or in the practice of other methods disclosed herein, or other conditions associated with or correlated to mechanical stress or a lack thereof.

Accordingly, the invention relates to compositions and methods for detecting and/or diagnosing osteoporosis or conditions or factors associated therewith, including genetic factors associated therewith.

Similarly, in the practice of the invention, e.g., protein detection, general methods in immunology may be employed. Standard methods in immunology known in the art and not specifically described are generally followed as in Stites et al.(eds), Basic and Clinical Immunology (8th Edition), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980). Immunoassays such as RIA and ELISA can be

employed to assess a specimen for the presence of specific proteins or other compounds of interest where appropriate as known in the art. Both polyclonal and monoclonal antibodies can be used in the assays. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor, New York, 1989

Antibodies may be used in various aspects of the invention, e.g., in detection or treatment or prevention methods. Antibodies may be either monoclonal, polyclonal or recombinant to be used in the immunoassays or other methods of analysis necessary for the practice of the invention. Conveniently, the antibodies may be prepared against the immunogen or portion thereof for example a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof may be isolated and used as the immunogen. The genes are identified as set forth in the present invention and the gene product identified. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, Antibody Engineering - A Practical Guide, W.H. Freeman and Co., 1992. Antibody fragments may also be prepared from the antibodies and include Fab, F(ab)₂, and Fv by methods known to those skilled in the art.

For producing polyclonal antibodies a host, such as a rabbit or goat, is immunized with the immunogen or immunogen fragment, generally with an adjuvant and, if necessary, coupled to a carrier; antibodies to the immunogen are collected from the sera. Further, the polyclonal antibody can be absorbed such that it is monospecific. That is, the sera can be absorbed against related immunogens so that no cross-reactive antibodies remain in the sera rendering it monospecific.

For producing monoclonal antibodies the technique involves hyperimmunization of an appropriate donor with the immunogen, generally a mouse, and isolation of splenic antibody producing cells. These cells are fused to a cell having immortality, such as a myeloma cell, to provide a fused cell hybrid which has

immortality and secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use.

For producing recombinant antibody (see generally Huston et al, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing Blymphocytes of animals, or hybridoma are reverse -transcribed to obtain complimentary DNAs (cDNAs). Antibody cDNA, which can be full or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art. (see for a general discussion Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992) The detectable moieties contemplated with the present invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, Beta-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{13}C and iodination.

Antibodies can also be used as an active agent in a therapeutic composition and such antibodies can be humanized, for instance, to enhance their effects. *See, e.g.* Huls et al., "A recombinant, fully human monoclonal antibody with antitumor activity constructed from phage-displayed antibody fragments," *Nature Biotechnology* Vol. 17, No. 3, March 1999, and documents cited therein, incorporated herein by reference.

Accordingly, antibodies from expression products of genes identified herein or by inventive methods disclosed herein are useful in immunodiagnostics, as well as in drugs or other commercial uses such as in research.

Simply, the expression product from the gene or portions thereof can be useful for generating antibodies such as monoclonal or polyclonal antibodies which are useful for diagnostic purposes or to block activity of expression products or portions thereof or of genes or a portion thereof, e.g., as a therapeutic. Monoclonal antibodies are immunoglobulins produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides greater specificity than a conventional, serum-derived antibody. Furthermore, screening a large number of monoclonal antibodies makes it possible to select an individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant, inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No. 4,196,265, issued Apr. 1, 1989, incorporated herein by reference, and other documents cited herein, e.g., *supra*.

Uses of monoclonal antibodies are known. One such use is in David, G. and Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983, incorporated herein by reference; *see also* documents cited herein, e.g., *supra*. Monoclonal antibodies have also been used to recover materials by immunoabsorption chromatography, *see, e.g.* Milstein, C., 1980, Scientific American 243:66, 70, incorporated herein by reference; and documents cited herein, such as *supra*. Thus, products expressed from genes identified herein or by methods herein or portions thereof are useful in therapeutics, immunoabsorption chromatography, as well as for generating antibodies for diagnostic or detection purposes. Furthermore, the expression products can be used in assays for detecting the presence of antibodies. For instance, the antibodies or expressed products can be used in assays analogous to those disclosed in U.S. Patents Nos. 5,591,645, 4,861,711, 5,861,319, 5,858,804, and 5,863,720, as well as in WO 86/04683, EP 154 749, WO 86/03839, and EP 186 799. Antibodies in the practice of the invention can include fragments thereof which are functional, e.g., a fragment that at least statistically significantly retains some (for instance a majority) or all of binding as compared with the entire antibody; for instance, antibodies comprehends a fragment comprising a binding domain.

Protein purification, including recombinant protein purification in the practice of the invention can be in accordance with or analogous to Marshak et al, "Strategies

for Protein Purification and Characterization. A laboratory course manual. " CSHL Press, 1996.

With respect to transgenic and knockout methods, the present invention comprehends transgenic gene and polymorphic gene animal and cellular (cell lines) models as well as for knockout models for the genes identified in the present invention. These models are constructed using standard methods known in the art and as set forth in United States Patents 5,487,992, 5,464,764, 5,387,742, 5,360,735, 5,347,075, 5,298,422, 5,288,846, 5,221,778, 5,175,385, 5,175,384, 5,175,383, 4,736,866 as well as Burke and Olson (1991), Capecchi (1989), Davies et al. (1992), Dickinson et al. (1993), Duff and Lincoln (1995), Huxley et al. (1991), Jakobovits et al. (1993), Lamb et al. (1993), Pearson and Choi (1993), Rothstein (1991), Schedl et al. (1993), Strauss et al. (1993) . Further, patent applications WO 94/23049, WO 93/14200, WO 94/06908, WO 94/28123 also provide information.

Thus, for instance, the inventive methods of the invention can be used to determine a gene of interest with respect to a physiological or disease state, e.g., osteoporosis or other conditions caused by mechanical stress for instance 608 or 405 or 274 and using the information herein and in the art (such as documents cited herein), knockout or transgenic animals such as mice or rat, can be prepared, to generate animals prone to the physiological or disease state, osteoporosis or other conditions caused by mechanical stress, to thereby test treatments or medicaments therefor; or, to test theories and thus advance research pertaining to the physiological or disease state, e.g., to test the functions of identified genes such as 405, 608 and 274, *inter alia*. Accordingly, from this disclosure and the knowledge in the art, no undue experimentation is needed to prepare knockout or transgenic animals, such as mice or rats or rodents; and, such animals have great value and utility.

Moreover, the genes of the present invention or a portion thereof, e.g., a portion thereof which expresses a protein which function the same as or analogously to the full length protein, or genes identified by the methods herein can be expressed recombinantly, e.g., in *E. coli* or in another vector or plasmid for either *in vivo* expression or *in vitro* expression. The methods for making and/or administering a vector or recombinant or plasmid for expression of gene products of genes of the invention or identified by the invention or a portion thereof either *in vivo* or *in vitro* can be any desired method, e.g., a method which is by or analogous to the methods

- disclosed in: U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, 4,722,848, WO 94/16716, WO 96/39491, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al., "Production of Human Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, U.S. application Serial No. 920,197, filed October 16, 1986, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman, "The function of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143 (recombinant adenovirus), Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also WO 98/33510; Ju et al., Diabetologia, 41:736-739, 1998 (lentiviral expression system); Sanford et al., U.S. Patent No. 4,945,050 (method for

transporting substances into living cells and tissues and apparatus therefor); Fischbach et al. (Intracel), WO 90/01543 (method for the genetic expression of heterologous proteins by cells transfected); Robinson et al., seminars in IMMUNOLOGY, vol. 9, pp.271-283 (1997) (DNA vaccines); Szoka et al., U.S. Patent No. 4,394,448 (method
5 of inserting DNA into living cells); and McCormick et al., U.S. Patent No. 5,677,178 (use of cytopathic viruses for therapy and prophylaxis of neoplasia).

The expression product generated by vectors or recombinants in this invention optionally can also be isolated and/or purified from infected or transfected cells; for instance, to prepare compositions for administration to patients. However, in certain
10 instances, it may be advantageous to not isolate and/or purify an expression product from a cell; for instance, when the cell or portions thereof enhance the effect of the polypeptide.

An inventive vector or recombinant expressing a gene identified herein or from a method herein or a portion thereof can be administered in any suitable amount to
15 achieve expression at a suitable dosage level, e.g., a dosage level analogous to the herein mentioned dosage levels (wherein the gene product is directly present). The inventive vector or recombinant can be administered to a patient or infected or transfected into cells in an amount of about at least 10^3 pfu; more preferably about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, for instance about 10^6 pfu
20 to about 10^8 pfu. In plasmid compositions, the dosage should be a sufficient amount of plasmid to elicit a response analogous to compositions wherein gene product or a portion thereof is directly present; or to have expression analogous to dosages in such compositions; or to have expression analogous to expression obtained *in vivo* by recombinant compositions. For instance, suitable quantities of plasmid DNA in
25 plasmid compositions can be 1 μ g to 100 mg, preferably 0.1 to 10 mg, e.g., 500 micrograms, but lower levels such as 0.1 to 2 mg or preferably 1-10 μ g may be employed. Documents cited herein regarding DNA plasmid vectors may be consulted for the skilled artisan to ascertain other suitable dosages for DNA plasmid vector compositions of the invention, without undue experimentation.

30 Compositions for administering vectors can be as in or analogous to such compositions in documents cited herein or as in or analogous to compositions herein described, e.g., pharmaceutical or therapeutic compositions and the like (e.g., *see infra*).

Thus, the invention comprehends *in vivo* gene expression which is sometimes termed "gene therapy". Gene therapy can refer to the transfer of genetic material (e.g. DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The particular gene that is to be used or which has been identified as the target gene is identified as set forth herein. The genetic material of interest encodes a product (e.g. a protein, polypeptide, peptide or functional RNA) whose production *in vivo* is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy cells are removed from a patient, and while being cultured are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, homologous recombination, etc.) and, an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to produce the transfected gene product *in situ*. In *in vivo* gene therapy, target cells are not removed from the subject rather the gene to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. Alternatively, if the host gene is defective, the gene is repaired *in situ* (Culver, 19981. These genetically altered cells have been shown to produce the transfected gene product *in situ*.

The gene expression vehicle is capable of delivery/transfer of heterologous nucleic acid into a host cell. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5' UTR and/or 3'UTR of the expression vehicle. Therefore as used herein the expression vehicle may, as needed, not include the 5'UTR and/or 3'UTR shown in sequences herein and only include the specific amino acid coding region.

The expression vehicle can include a promotor for controlling transcription of the heterologous material and can be either a constitutive or inducible promotor to allow selective transcription. Enhancers that may be required to obtain necessary

transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene as described herein.

- 5 Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and Gilboa et al (1986), as well as other documents cited herein (*see supra*) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.
- 10 In addition, see United States patent 4,866,042 for vectors involving the central nervous system and also United States patents 5,464,764 and 5,487,992 for positive-negative selection methods.

- Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature.
- 20 Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types in vivo or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

- 25 A specific example of DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most
- 30 cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions, can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that
5 confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to
10 particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example,
15 retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are
20 unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes
25 its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art. For example, if breast cancer is to be treated then a vector specific for such epithelial cells would be used. Likewise, if diseases or
30 pathological conditions of the hematopoietic system are to be treated, then a viral vector that is specific for blood cells and their precursors, preferably for the specific type of hematopoietic cell, would be used.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles which are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The recombinant vector can be administered in several ways. If viral vectors are used, for example, the procedure can take advantage of their target specificity and consequently, do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of neurodegenerative diseases. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors,

viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

5 Inventive vectors can comprise a herein defined gene, as well as a regulatory element operative linked thereto, e.g., a promoter, for expression; and, the regulatory element or promoter can be tissue or cell specific; for instance, the regulatory element or promoter can be for expression in a cell or precursor thereto employed in an inventive or herein-described or herein-cited test, e.g., the regulatory element or
10 promoter can be for expression in a bone cell such as an osteoblast or an osteoclast or a precursor thereto.

 Delivery of gene products (products from herein defined genes: genes identified herein or by inventive methods or portions thereof) and/or antibodies or portions thereof and/or agonists or antagonists (collectively or individually
15 "therapeutics"), and compositions comprising the same, as well as of compositions comprising a vector expressing gene products, can be done without undue experimentation from this disclosure and the knowledge in the art.

 The present invention provides compositions comprising an expression vector comprising a herein defined gene, or a portion thereof, e.g., which codes for a
20 functional portion thereof, as well as therapeutics based on the genes identified herein, e.g., compositions comprising expression products or a functional portion thereof or antibodies thereto or a functional portion thereof and/or agonists or antagonists. (Thus, a herein defined gene can comprehend a portion thereof which expresses a functional portion of a full length expression product.). The therapeutics and vectors
25 of the present invention are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight, species of the patient, and other factors known to those skilled in the pharmaceutical or veterinary arts.

30 The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other

indicators, e.g., of osteoporosis, for instance, improvement in bone density, as are selected as appropriate measures by those skilled in the art.

Where appropriate the therapeutics of the present invention are pharmaceuticals and as such can be administered in various ways. It should be noted
5 that these therapeutics can be administered as the expression product and/or portion thereof and/or antibody and/or portion thereof or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles, as well as other active ingredients (e.g., other expression products, portions thereof, antibodies,
10 portions thereof, from inventive methods, and/or other therapies, such as those discussed herein). The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques.

Implants of the therapeutics and/or of vectors expressing the herein defined
15 genes are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention. The implant can be placed near bone, to
20 stimulate bone growth or increase bone density. With respect to implants or slow release systems that can be used in the practice of the invention with respect to therapeutics, or vectors expressing the herein defined genes, mention is made of U.S. Patents Nos. 4,150,108, 4,329,332, 4,331,652, 4,333,919, 4,389,330, 4,489,055, 4,526,938, 4,530,840, 4,542,025, 4,563,489, 4,675,189, 4,677,191, 4,683,288,
25 4,758,435, 4,857,335, 4,931,287, 5,178,872, 5,252,701, 5,275,820, 5,478,564, 5,540,912, 5,447,725, 5,599,852, 5,607,686, 5,609,886, 5,631,015, 5,654,010, 5,700,485, 5,702,717, 5,711,968, 5,733,566, 4,938,763, 5,077,049, 5,278,201, 5,278,202, 5,288,496, 5,324,519, 5,324,520, 5,340,849, 5,368,859, 5,401,507, 5,419,910, 5,427,796, 5,487,897, 5,599,552, 5,632,727, 5,643,595, 5,660,849,
30 5,686,092, 5,702,716, 5,707,647, 5,717,030, 5,725,491, 5,733,950, 5,736,152, 5,744,153, 5,759,563, and 5,780,044, European Patent Application 0537559, Shah et al (J. Controlled Release, 1993, 27:139-147), Lambert and Peck (J. Controlled

Release, 1995, 33:189-195), and Shivley et al (J. Controlled Release, 1995, 33:237-243).

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred. Thus, one can scale up from animal experiments, e.g., rats, mice, and the like, to humans, by techniques from this disclosure and the knowledge in the art, without undue experimentation.

The doses may be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.

When administering a therapeutic or vector of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions

Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate

and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention, e.g., comprising a therapeutic and/or vector, can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include: 5, 225, 182; 5, 169, 383; 5, 167, 616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art (*See also* documents cited herein, e.g., *supra*).

A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver it orally or intravenously and retain the biological activity are preferred.

In one embodiment, a formulation of the present invention can be administered initially, and thereafter maintained by further administration. For instance, a formulation of the invention can be administered in one type of composition and thereafter further administered in a different or the same type of composition. For example, a formulation of the invention can be administered by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used.

The quantity to be administered will vary for the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 pg/kg to 10 mg/kg per day. For instance, dosages can be

readily ascertained by those skilled in the art from this disclosure and the knowledge in the art. Thus, the skilled artisan can readily determine the amount of gene product and optional additives, vehicles, carrier and/or adjuvant in compositions and to be administered in methods of the invention. Typically, an adjuvant or additive is commonly used as 0.001 to 50 wt% solution in phosphate buffered saline, and the gene product or active ingredient is present on the order of micrograms to milligrams, such as about 0.0001 to about 5 wt%, preferably about 0.0001 to about 1 wt%, most preferably about 0.0001 to about 0.05 wt% or about 0.001 to about 20 wt%, preferably about 0.01 to about 10 wt%, and most preferably about 0.05 to about 5 wt%. Of course, for any composition to be administered to an animal or human, including the components thereof, and for any particular method of administration, it is preferred to determine therefor: toxicity, such as by determining the lethal dose (LD) and LD₅₀ in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response, such as by titrations of sera and analysis thereof, e.g., by ELISA and/or RFFIT analysis. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

Examples of compositions comprising a therapeutic and/or vector of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, peroral, intragastric, mucosal (e.g., perlingual, alveolar, gingival, olfactory or respiratory mucosa) etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition,

1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Compositions of the invention, are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions or viscous compositions which may be buffered to a selected pH. If digestive tract absorption is preferred, compositions of the invention can be in the "solid" form of pills, tablets, capsules, caplets and the like, including "solid" preparations which are time-released or which have a liquid filling, e.g., gelatin covered liquid, whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or, a dose having a particular particle size.

Compositions of the invention can contain pharmaceutically acceptable flavors and/or colors for rendering them more appealing, especially if they are administered orally. The viscous compositions may be in the form of gels, lotions, ointments, creams and the like (e.g., for transdermal administration) and will typically contain a sufficient amount of a thickening agent so that the viscosity is from about 2500 to 6500 cps, although more viscous compositions, even up to 10,000 cps may be employed. Viscous compositions have a viscosity preferably of 2500 to 5000 cps, since above that range they become more difficult to administer. However, above that range, the compositions can approach solid or gelatin forms which are then easily administered as a swallowed pill for oral ingestion.

Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection or orally, to animals, children, particularly small children, and others who may have difficulty swallowing a pill, tablet, capsule or the like, or in multi-dose situations. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with mucosa, such as the lining of the stomach or nasal mucosa.

Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g.,

liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form), or solid dosage form (e.g., whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form or liquid-filled form).

5 Solutions, suspensions and gels, normally contain a major amount of water (preferably purified water) in addition to the antigen, lipoprotein and optional adjuvant. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents, jelling agents, (e.g., methylcellulose), colors and/or flavors may also be
10 present. The compositions can be isotonic, i.e., it can have the same osmotic pressure as blood and lacrimal fluid.

 The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or
15 organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

 Viscosity of the compositions may be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable
20 thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

25 As mentioned herein, a pharmaceutically acceptable preservative can be employed to increase the shelf-life of the compositions. Benzyl alcohol may be suitable, although a variety of preservatives including, for example, parabens, thimerosal, chlorobutanol, or benzalkonium chloride may also be employed. A suitable concentration of the preservative will be from 0.02% to 2% based on the total
30 weight although there may be appreciable variation depending upon the agent selected.

 Those skilled in the art will recognize that the components of the compositions should be selected to be chemically inert with respect to the gene product and optional

adjuvant or additive. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

5 The inventive compositions of this invention are prepared by mixing the ingredients following generally accepted procedures. For example the selected components may be simply mixed in a blender, or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control
10 pH or an additional solute to control tonicity. Generally the pH may be from about 3 to 7.5. Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient or animal, and the composition form used for administration (e.g., solid vs. liquid). Dosages for
15 humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, the Examples below.

 Suitable regimes for initial administration and booster doses or for sequential administrations also are variable, may include an initial administration followed by subsequent administrations; but nonetheless, may be ascertained by the skilled artisan,
20 from this disclosure, the documents cited herein, and the Examples below.

 Accordingly, the invention comprehends, in further aspects, methods for preparing therapeutic compositions including a gene product or functional fragment thereof of a gene identified herein or a gene identified in an inventive method herein, as well as to methods for increasing bone density, treating, preventing or controlling
25 osteoporosis, or otherwise alleviating a condition caused by mechanical stress or inducing bone growth, comprising administering an inventive composition, or a gene product, or functional fragment thereof of a gene identified herein or a gene identified in an inventive method herein, or a vector expressing such a gene.

 In this context and as used throughout this specification, "functional" means a
30 protein having part or all of the primary structural conformation of the protein gene product of a gene identified herein or of a gene identified by the methods herein, and possessing the biological property of contributing to the development of bone cells in the same or an analogous fashion to the full length protein gene product, said protein

gene product being either isolated from a natural source or being the product of procaryotic or eukaryotic expression or of protein synthesis methods. The protein can have an amino acid sequence comprising an amino acid sequence of a sequence disclosed herein or of a gene product of a gene identified by a method herein or any
5 fragment or derivative thereof by way of amino acid deletion, substitution, insertion, addition and/or replacement of the amino acid sequence. Also comprised by the term "functional" protein is the capability of said protein or part thereof to generate a specific immune response such as an antibody response; e.g., to bind to antibodies elicited by the full length protein.

10 Moreover, the present invention and embodiments thereof provide advances in and assist to further research and knowledge with respect to osteoporosis and conditions caused by or having as a factor mechanical stress or a lack thereof and provide an insight into development and maintenance of bone tissue. The present invention and embodiments thereof also provide advances in and assist to further
15 clinical and epidemiological research, e.g., to allow others to further explore and extend the current potential for practical prevention and treatment. Further still, the present invention and embodiments thereof provide a deeper knowledge of factors controlling bone cell activity and regulation of bone mineral and matrix formation and remodeling contribute ultimately to the understanding of the etiology of osteoporosis
20 or other conditions involving mechanical stress or a lack thereof. For example, the present invention provides osteoporosis or mechanical stress or lack thereof models for *in vitro* studies. This understanding will permit a more rational choice and evaluation of therapies, even as current treatments are evaluated clinically. Moreover, the present invention, for instance, via the inventive models, allows for the discovery of
25 genes involved in processes of osteoporosis and/or one growth or formation or bone cell activity, *inter alia*. Every new gene discovered sheds more light on the complex molecular events that govern all aspects of life. The elucidation of the function of the gene and its place and role in this intricate network of pathways and structures resolves another piece in the puzzle of life. Thus, the educational and research
30 implications are very clear. Sometimes genes may have much more benefit in this respect than in the therapeutics/diagnostics fields.

A better understanding of the present invention and of its many advantages will be had from the following examples, given by way of illustration and as a further description of the invention.

EXAMPLES/RESULTS

5 **Example/Result 1: ANALYSIS OF GENES AT A
TRANSCRIPTIONAL LEVEL
USING NUCLEAR mRNA PROBES**

DIFFERENTIAL TRANSLATION

10 MATERIALS/METHODS (WHICH MAY APPLY IN
WHOLE OR PART TO SOME OR ALL EXAMPLES)

General Scheme

- a. Total mRNA organic extraction of all RNA from the source tissue or cell.
(additional selection for polyA+ mRNA can be included).
- b. Nuclear RNA-lysis of cells (from a tissue or a cell line) by homogenization in
15 hypotonic buffer. Collection of nuclei by centrifugation and organic extraction of the
RNA.
- c. Cytoplasmic RNA - Organic extraction of the RNA from the supernatant from b
above.
- d. Polyribosomal/subpolyribosomal fractionation. Lysis of cells by homogenization
20 hypotonic buffer, removal of nuclei and fractionation of polyribosome on linear
sucrose gradients and organic extraction of the RNA from each fraction of the
gradient.
- e. Secreted and membrane encoding transcripts.
 1. Isolation of RER on Percoll gradients (after homogenization of cells).
 - 25 2. Preparation of microsomes containing the RER
 3. Isolation of membrane-bound polyribosomes by successive treatment of cells
with detergents.
- f. Nuclear proteins. Isolation of cytoskeletal associated polyribosomes by treating
cells lyzates with different detergents.
- 30 g. Mitochondrial genes. Isolation of mitochondria on Percoll gradients.
- h. Alternative splicing. Separation of nuclei and isolation of splicosome (proteins
and RNA complex) on linear sucrose gradients.

Preparation of cell extracts: Cells were centrifuged. The pellet was washed
with PBS and recentrifuged. The cells were resuspended in 4x of one packed cell

volume (PCV) with hypotonic lysis buffer (HLB: 20mM TrisHCL pH=7.4; 10mM NaCl; 3mM MgCl₂). The cells were incubated five minutes on ice. 1xPCV of HLB containing 1.2% Triton X-100 and 0.2M sucrose was added. The cells were homogenized with a Dounce homogenizer (five strokes with B pestle). The cell lysate
5 was centrifuged at 2300g for ten minutes at 4°C. The supernatant was transferred to a new tube. HLB containing 10mg/ml heparin was added to a final concentration of 1mg/ml heparin. NaCl was added to a final concentration of 0.15M. The supernatant was frozen at
-70°C after quick freezing in liquid N₂ or used immediately.

10 Sucrose gradient fractionation: A linear sucrose gradient from 0.5M to 1.5M sucrose in HLB was prepared. Polyallomer tubes (14X89mm) were used. 0.5 to 1.0ml of cell extract was loaded on the gradient. The cells were centrifuged at 36,000 RPM for 110 minutes at 4°C. An ISCO Density Fractionator was used to collect the fractions and record the absorbance profile.

15 RNA purification: SDS was added to 0.5% and Proteinase K to 0.1mg/ml and incubated at 37°C for 30 minutes. Extract with an equal volume of phenol+chloroform (1:1). The aqueous phase was extracted with one volume of chloroform and the RNA was precipitated by adding Na-Acetate to 0.3M and 2.5 volumes of ethanol and incubating at -20°C overnight. Centrifuged ten minutes, the
20 supernatant was aspirated and the RNA pellet was dissolved in sterile, diethylpyrocarbonate (hereinafter referred to as "DEPC") DEPC-treated water.

Preparation of Microsomes: When possible fresh tissues and cells are used, without freezing. Tissues were powdered in liquid nitrogen with mortar and pestle and then homogenized using 4ml of buffer A/1 gr tissue (Buffer A is 250mM sucrose,
25 50mM TEA, 50mM KOAc pH7.5, 6mM Mg(Oac)₂, 1mM EDTA, 1mM DTT, 0.5mM PMSF. PMSF was made in ethanol before making the buffer and added in drops to buffer while being stirred. This was stirred for 15 minutes and then DTT was added). Fresh organs were washed in Buffer A a few times, and then cut into pieces and homogenized. Approximately 5ml buffer A/5x10⁸ cells were added and
30 homogenized. This was then homogenized on ice for 5-10 times, or as needed with the individual tissue. The mixture was transferred to 50ml tubes, then centrifuged for 10 minutes, at 4°C in a swinging bucket rotor machine. Next, the supernatant was transferred, avoiding the pellet as much as possible, to a Sorvall tube, the pellet was

washed again with 1ml buffer and centrifuge as before. The two pellets were combined, thus establishing the nuclear fraction. The combination was dissolved and treated the pellet with Tri-reagent (usually 2ml of Tri-reagent when sample is from cells) to extract the nuclear RNA. The combined 1st and 2nd supernatants were

5 centrifuged for 10 minutes at 10000g at 4°C. Again, the supernatant was transferred to a tube and kept on ice. The pellet was washed again with 1ml buffer and centrifuged for 10 minutes at 10000g and the two pellets were combined as before, thus establishing the Mitochondrial pellet. Again, the pellet was treated with Tri-reagent (usually 1ml with cells) and the Mitochondrial RNA was extracted. Next,

10 cold ultracentrifuge tubes were prepared containing a sucrose cushion made of: buffer A + 1.3M sucrose. The volume of the cushion was approximately 1/3 of the supernatant. The supernatant was loaded on the cushion in a 1:3 ratio of cushion to supernatant. A pair of tubes was weighed for balancing, a 20-30mg difference is allowable. The tubes were centrifuged 2.5 hours at 140,000g, 4°C with a Ti60.2 rotor

15 (45,000 rpm). When two phases of supernatant were visible, then the red phase only was transferred (if possible), as the cytoplasmic fraction, to a sorvall tube. The clear supernatant was aspirated. When not possible to separate or phase distinction not visible, all the supernatant was taken as cytoplasmic fraction and dilute sucrose with TE (10mM Tris-HCl pH 8.0, 1mM EDTA). In the pellet were the microsomes which

20 were visible and were clear or yellowish. For the RNA extraction, the cytoplasmic fraction was treated with 1% SDS, 0.1mg/ml proteinase K, for 30 minutes, at 37°C. After this, freezing at -80°C was possible. The RNA was extracted with a phenol:chloroform combination and precipitate with 0.3M Na-acetate, 1µl glycogen, and equal volume of isopropanol. O'N precipitation was possible and can be

25 accomplished at 30 minutes on ice. The extract was spun at 10000g, for 20 minutes, then the RNA pellet was washed with 70% ethanol. The pellet was dried and then dissolved in H₂O. The microsomes were then dissolved with 0.1M NaCl/1% SDS solution (1ml is usually sufficient for a small pellet) and extracted with a phenol:chloroform combination (no proteinase K treatment). Then the precipitation

30 of the RNA was done in the same way as for the cytoplasmic fraction but without the requirement of adding salt.

Preparation of Nuclear and Cytoplasmic RNA: Subconfluent plates were washed with 125 mM KCl-30 mM Tris-hydrochloride (pH 7.5)-5 mM magnesium

acetate-1 mM 2-mercaptoethanol-2 mM ribonucleoside vanadyl complex (2)-0.15 mM spermine-0.05 mM spermidine at 4°C, and cells scraped from the plates were washed twice with the same buffer. Approximately 10⁸ cells were allowed to swell for 10 minutes in 2.5 ml of swelling buffer (same as wash buffer except the KCl concentration was 10 mM) lysed with 20 strokes of a Dounce homogenizer (B pestle), overlaid on an equal volume of swelling buffer containing 25% glycerol, and centrifuged for 5 min. at 400 x g and 4°C. The upper layer of the supernatant, which contained 90% of the CAD sequences released by lysis, was designated the cytoplasmic fraction. The nuclear pellet was washed once with 2 ml of swelling buffer-25% glycerol-0.5% Triton X-100 and once with 2 ml of swelling buffer.

Nuclear RNP. Nuclei from 10⁸ cells, prepared as described above, were suspended in 1 ml of 10 mM Tris-hydrochloride (pH 8.0)-100 mM NaCl-2 mM MgCl₂-1 mM 2-mercapthoethanol-0.15 mM spermine-0.05 mM spermidine-10 mM ribonucleoside vanadyl complex (2)-100 U of placental RNase inhibitor (Amersham Corp.) per ml and sonicated at the maximum power setting of a Konres micro-ultrasonic cell disrupter for 20 g at 4°C. Bacterial tRNA (2 mg) was added, to adsorb basic proteins (9), and the mixture was centrifuged for 1 minute (Eppendorf microcentrifuge). The supernatant was applied to a 15 to 45% sucrose gradient in mM Tris-hydrochloride-100 mM NaCl-2 mM MgCl₂-2 mM ribonucleoside vanadyl complex and centrifuged in a Beckman SW41 rotor for 90 minutes at 40,000 rpm and 4°C. RNA was recovered from gradient fractions by the addition of sodium dodecyl sulfate to 0.5%, treatment with proteinase K (200 µg/ml) for 2 hours at 37°C, extraction with phenol, and precipitation with ethanol.

Preparation of Antisense RNA: Total cellular RNA is extracted. Part of the RNA pool is immobilized on a membrane, another part converted into cDNA after ligation of oligodeoxynucleotides to the 3'-ends. The use of biotinylated, complementary oligos for cDNA synthesis allows immobilization of a "minus" strand to streptavidin-coated magnetic beads. A second set of oligos is ligated to the cDNA at the previous 5'-end of the RNA. Plus strands are eluted from the bound strands and hybridized to the membrane-bound RNA. Since the cDNA strand used has the same polarity of the RNAs, only cDNA sequences that can bind to complementary RNAs should be retained. PCR amplification and subsequent cloning of PCR-fragments is followed by sequence analysis. To test whether cloned sequences are correctly

identified, probes are generated in sense and antisense direction. Positive clones will be structurally and functionally characterized. In order to work out this method, we started using a bacterial strain (*Escherichia coli*), containing plasmid R1 that regulates its copy number by antisense RNA. Previous work has identified both antisense
5 (CopA) and target RNA (CopT) of R1 intracellularly. This procedure, if feasible, will then be used to screen for antisense RNA systems in other organisms.

DIFFERENTIAL ANALYSIS

Differential display: Reverse transcription: 2µg of RNA were annealed with 1pmol of oligo dT primer (dT)₁₈ in a volume of 6.5µl by heating to 70°C for five
10 minutes and cooling on ice. 2µl reaction buffer (x5), 1µl of 10mM dNTP mix, and 0.5µl of SuperScript II reverse transcriptase (GibcoBRL) was added. The reaction was carried out for one hour at 42°C. The reaction was stopped by adding 70µl TE (10mM Tris pH=8; 0.1mM EDTA). Oligonucleotides used for Differential display: The oligonucleotides were essentially those described in the Delta RNA
15 Fingerprinting kit (Clontech Labs. Inc.). There were 9 "T" oligonucleotides of the structure: 5' CATTATGCTGAGTGATATCTTTTTTTTXY 3' (SEQ ID No:). The 10 "P" oligonucleotides were of the structure: 3' ATTAACCCTCACTAAA "TGCTGGGGA" 3' (SEQ ID No:) where the 9 or 10 nucleotides between the parenthesis represent an arbitrary sequence and there are 10 different sequences (SEQ
20 ID Nos.), one for each "P" oligo.

Amplification reactions: each reaction is done in 20µl and contains 50µM dNTP mix, 1µM from each primer, 1x polymerase buffer, 1 unit expand Polymerase (Boehringer Mannheim), 2µCi [α -³²P]dATP and 1µl cDNA template. Cycling
25 conditions were: three minutes at 95°C, then three cycles of two minutes at 94°C, five minutes at 40°C, five minutes at 68°C. This was followed by 27 cycles of one minute at 94°C, two minutes at 60°C, two minutes at 68°C. Reactions were terminated by a seven minute incubation at 68°C and addition of 20µl sequencing stop solution (95% formamide, 10mM NaOH, 0.025% bromophenol blue, 0.025% xylene cyanol).

Gel analysis: 3-4µl were loaded onto a 5% sequencing polyacrylamide gel
30 and samples were electrophoresed at 2000 volts/40 milliamperes until the slow dye (xylene cyanol) was about 2 cm from the bottom. The gel was transferred to a filter paper, dried under vacuum and exposed to x-ray film.

Recovery of differential bands: bands showing any a differential between the various pools were excised out of the dried gel and placed in a microcentrifuge tube. 50 μ l of sterile H₂O were added and the tubes heated to 100°C for five minutes. 1 μ l was added to a 49 μ l PCR reaction using the same primers used for the differential display and the samples were amplified for 30 cycles of: one minute at 94°C, one minute at 60°C and one minute at 68°C. 10 μ l was analyzed on agarous gel to visualize and confirm successful amplification.

REPRESENTATIONAL DIFFERENCE ANALYSIS

Reverse transcription: as above but with 2 μ g polyA+ selected mRNA.

- 10 Preparation of double stranded cDNA: cDNA from previous step was treated with alkali to remove the mRNA, precipitated and dissolved in 20 μ l H₂O. 5 μ l buffer, 2 μ l 10mM dATP, H₂O to 48 μ l and 2 μ l terminal deoxynucleotide transferase (TdT) were added. The reaction was incubated 2-4 hours at 37°C. 5 μ l oligo dT (1 μ g/ μ l) was added and incubated at 60°C for 5 minutes. 5 μ l 200 mM DTT, 10 μ l 10x section
- 15 buffer (100mM Mg Cl₂, 900 mM Hepes, pH 6.6) 16 μ l dNTPs (1 mM), and 16 U of Klenow were added and the mixture was incubated overnight at room temperature to generate ds cDNA. 100 μ l TE was added and extracted with phenol/chloroform. The DNA was precipitated and dissolved in 50 μ l H₂O.

- Generation of representations: cDNA with DpnII was digested by adding 3 μ l
- 20 DpnII reaction buffer 20 V and DpnII to 25 μ l cDNA and incubated five hours at 37°C. 50 μ l TE was added and extracted with phenol/chloroform. cDNA was precipitated and dissolved to a concentration of 10ng/ μ l.

The following oligonucleotides are used in this procedure:

- R-Bgl-12 5' GATCTGCGGTGA 3' (SEQ ID No:)
- 25 R-Bgl-24 5' AGCACTCTCCAGCCTCTCACCGCA 3' (SEQ ID No:)
- J-Bgl-12 5' GATCTGTTTCATG 3' (SEQ ID No:)
- J-Bgl-24 5' ACCGACGTCGACTATCCATGAACA 3' (SEQ ID No:)
- N-Bgl-12 5' GATCTTCCCTCG 3' (SEQ ID No:)
- N-Bgl-24 5' AGGCAACTGTGCTATCCGAGGGAA 3' (SEQ IDNo:)
- 30 R-Bgl-12 and R-Bgl-24 oligos were ligated to Tester and Driver: 1.2 μ g DpnII digested cDNA. 4 μ l from each oligo and 5 μ l ligation buffer X10 and annealed at 60°C for ten minutes. 2 μ l ligase was added and incubated overnight at 16°C. The

ligation mixture was diluted by adding 140 μ l TE. Amplification was carried out in a volume of 200 μ l using R-Bgl-24 primer and 2 μ l ligation product and repeated in twenty tubes for each sample. Before adding Taq DNA polymerase, the tubes were heated to 72°C for three minutes. PCR conditions were as follows: five minutes at 72°C, twenty cycles of one minute at 95°C and three minutes at 72°C, followed by ten minutes at 72°C.

Every four reactions were combined, extracted with phenol/chloroform and precipitated. Amplified DNA was dissolved to a concentration of 0.5 μ g/ μ l and all samples were pooled.

Subtraction: Tester DNA (20 μ g) was digested with DpnII as above and separated on a 1.2% agarous gel. The DNA was extracted from the gel and 2 μ g was ligated to J-Bgl-12 and J-Bgl24 oligos as described above for the R-oligos. The ligated Tester DNA was diluted to 10ng/ μ l with TE. Driver DNA was digested with DpnII and repurified to a final concentration of 0.5 μ g/ μ l. Mix 40 μ g of Driver DNA with 0.4 μ g of Tester DNA. Extraction was carried out with phenol/chloroform and precipitated using two washes with 70% ethanol, resuspended DNA in 4 μ l of 30mM EPPS pH=8.0, 3mM EDTA and overlaid with 35 μ l mineral oil. Denatured at 98°C for five minutes, cool to 67°C and 1 μ l of 5M NaCl was added to the DNA. Incubated at 67°C for twenty hours. Diluted DNA by adding 400 μ l TE.

Amplification: Amplification of subtracted DNA in a final volume of 200 μ l as follows: Buffer, nucleotides and 20 μ l of the diluted DNA were added, heated to 72°C, and Taq DNA polymerase was added. Incubated at 72°C for five minutes and added J-Bgl-24 oligo. Ten cycles of one minute at 95°C, three minutes at 70°C were performed. Incubated ten minutes at 72°C. The amplification was repeated in four separate tubes. The amplified DNA was extracted with phenol/chloroform, precipitated and all four tubes were combined in 40 μ l 0.2XTE, Digested with Mung Bean Nuclease as follows: To 20 μ l DNA 4 μ l buffer, 14 μ l H₂O and 2 μ l Mung Bean Nuclease (10 units/ μ l) was added. Incubated at 30°C for thirty-five minutes + First Differential Product (DPI).

Repeat subtraction hybridization and PCR amplification at driver: differential ratio of 1:400 (DPII) and 1:40,000 (DPIII) using N-Bgl oligonucleotides and J-Bgl

oligonucleotides, respectively. Differential products were cloned into a Bluescript vector at the BAM HI site for analysis of the individual clones.

The experimental cells were grown alternatively under normal conditions, for 4 hours under hypoxia (<1% oxygen) and for 16 hours under hypoxia. The cells were
5 harvested and RNA was extracted either from nuclei that were prepared from the cells (nuclear RNA) or from extracts of unfractionated cells (total cellular RNA).

Figure 2 demonstrates how the probes prepared from the nuclear RNA (STP) give a higher differential expression than the total cellular RNA probe (Tot). The control genes encoding VEGF (vascular endothelial growth factor), Glut1 (glucose
10 transporter 1) and glycogen synthase are known to be induced by the hypoxia stress. The level of induction observed in the nuclear probe is much higher than that seen in the total probe and much closer to the actual know level of induction. The three new genes RTP 241, RTP 262 and RTP 779 show marked induction by hypoxia. Again, the induction level seen with the nuclear probe is much higher, up to five-fold higher,
15 as seen for RTP779. When the induction of these genes was analyzed by the Northern blot method, it was found that the nuclear probe was once again much closer to the actual situation, while the total probe gives a marked underestimation.

The genes RTPi-66 and RTP2I-72 demonstrate the ability of the nuclear probe to detect differentially expressed genes that do not appear differentially with the total
20 probe.

The genes for Nucleolin and Thrombospondin show that also for down-regulated mRNAs the nuclear probe is much more sensitive and gives much high levels of differential expression values.

Lastly, the genes for ribosomal protein L17 and cytoplasmic gamma-actin are
25 known as genes that do not respond to hypoxia stress. The nuclear probe and the total probe both show that no induction occurs.

Example/Result 2: DIFFERENTIAL EXPRESSION PROFILING

Chip: The microarray (Chip) used was prepared as follows. Subtraction experiments

30 were carried out on rat osteoblasts (Calvaria) using CLONTECH SSH kit (K 1804- 1). Cells were subjected to 20 minutes of mechanical stress and compared to "normal" cells not subjected to mechanical force. 767

76

induced sequences and 606 reduced sequences were selected and printed on a chip.

Probe: Total RNA

Cells: Primary Calvaria cultures derived from 17-19 days old rat embryos.

5 **List of Analyses:**

CHIP no	Experiment	Analysis
109	3 -Ca +/-mechanical force	Compare the system with and without application of mechanical force in absence of Ca in culture medium. Find genes differentially expressed under the influence of mechanical force.
110	2 +Ca +/-mechanical force	Compare the system with and without application of mechanical force in presence of Ca in culture medium. Find genes differentially expressed under the influence of mechanical force.
111	1 +Indomethacin +/- mechanical force	Compare the system with and without application of mechanical force. Find genes differentially expressed under the influence of mechanical force (with prostaglandin synthesis inhibited by indomethacin).
107	4 +/- PEG2	Compare the system with and without PGE2 treatment (to mimic mechanical force)
116	5 +/- PEG2	Compare the system with and without PGE2 treatment (to mimic mechanical force)

Calvaria treated with indomethacin and mechanical force

Primary cell cultures derived from 17-19 days old rat embryos. The cultures were prepared by trypsin - EDTA digestion of Calvaria including the periosteum. The cell cultures were grown in MEM medium with 10% FCS for 5-6 days to reach confluency.

At this time 10 microliter which contains 20 μ g of indomethacin were added to culture dishes which had 4ml of medium. 20 minutes later the dishes were activated mechanically. The mechanical activation is carried out e.g. by expanding an orthodontic expansion screw which is attached to two pieces of solid acrylic resin

glued to the outer surface of the cell culture dish. The expansion deforms the dish irreversibly. Same cultures which were not treated with indomethacin and activated mechanically were as positive control.

The rationale is because mechanical activation stimulates *de novo* synthesis of prostaglandins. Indomethacin inhibits synthesis of prostaglandins.

Calvaria. grown in the presence of Ca activated by mechanical force

Calvaria grown in the presence of Ca were activated by mechanical activating devices at confluency. The cultures were prepared as described above. The cells were grown in MEM medium which normally includes 1 mM of calcium: - from the seeding of the cells until confluency and mechanical activation.

Calvaria grown in absence of Ca treated with mechanical force

The cultures were grown in MEM medium which was calcium free. The calcium in this medium was 0.25 mM because it consisted of 10% FCS (serum contains 2.5 mM of calcium). After 3-4 days the medium was replaced by regular MEM which included normal calcium concentration.

The rationale of this experiment is mainly because transudation mechanism of mechanical activation like prostaglandin synthesis and action is calcium dependent. The cultures in low calcium suppress the proliferation of fibroblasts and allow growth and differentiation of osteoblasts in culture. Therefore, the strategy is to start with low calcium medium and after 3-4 days to booster growth by switching to normal calcium medium (1 mM Ca).

Calvaria treated with prostaglandin PGE2

Primary Calvaria cells were treated with PGE2 in both experiments (both are similar primary cultures prepared at different date, and treated identically).

PGE2 treatment was performed on cultures which reached confluency by adding (treating with) 10 microliter of PGE2 which consists of total of 500ng of PGE2. After 30 minutes the cells were scraped and stored in -70°C.

The rationale: The PGE2 treatment is supposed to mimic the mechanical activation effect.

The results from this Example are shown in the Table and sequences of Figure 2A. Novel sequences CMF608, CMF405 and CMF274 were identified, *inter alia*, as discussed below (see also Figs 2A-14).

AN OSTEOPOROSIS (or mechanical stress) MODEL (calvaria cell cultures).**Differentially regulated/differentially expressed genes post-mechanical stimulation****Extracellular matrix, transmembranal and secreted proteins:**

- 5 tenascin
- collagen XII
- thrombospondin 1
- ADAMTS-1.
- C3 complement component
- 10 alpha-2-macroglobulin receptor
- fibronectin
- connective tissue growth factor
- endothelin converting enzyme
- alpha-2u microglobulin-related protein
- 15 RB13-6

Genes connected to regulation of apoptosis

SARP1
cytochrome oxidase subunit 1
glutamyl-cystein synthetase

- 20 **Genes connected to intracellular fatty acid methabolism**
- 3-hydroxy-3-methylglutaryl coenzyme A reductase
- yeast ERG3 homologue
- and yeast ERG25 homologue
- stearoyl-CoA desaturase

- 25 **Genes connected to cytoskeleton regulation**

AHNAK
filamin
syntrophin 1

Genes connected to regulation of water channels

- 30 aquaporin1

Novel genes or known anonymous genes without function

- highly charged amino acid sequence
- DEST274 (CMF274; see Figs 2A-14)
- DEST405 (CMF405; see Figs 2A-14)
- 35 DEST608 (CMF 608; see Figs 2A-14)

General overview of identified genes.

- Tenascin is an extracellular matrix glycoprotein whose expression is up-regulated in normal bone development during condensation. It is also involved in genesis and function of articular chondrocytes. Tenascin is secreted by osteoblasts,
- 40 but is absent from mineralized bone matrix. Expression of alkaline phosphatase

activity and **collagen XII** (markers of osteoblast differentiation) are tenascin-dependent (down-regulated by anti-tenascin RNA). Expression of tenascin is markedly increased in response to mechanical stress, its promoter (in chicken) was shown to contain a cis-acting "strain-responsive" element.

- 5 Another protein whose expression is known to be modulated by mechanical stress (fluid shear or stretched stress of i.e. mesothelial cells) is endothelin (**endothelin converting enzyme** that generates active endothelin molecules from inactive intermediates is upregulated in the present screen). In bone, endothelin stimulates the osteoblastic IL-1-induced production of IL-6 - mediator of osteoclastic
- 10 differentiation, function and probably survival. Receptors to endothelin were demonstrated in osteoblastic cells by ligand binding (autocrine loop). Major endothelin signal transduction pathways in bone cells is stimulation of phospholipid turnover, by activation of phospholipases A, C, and D, stimulation of Ca flux from intra- and extracellular stores and activation of tyrosine kinases. Endothelins also
- 15 modulated calcium signaling elicited by other agents (i.e. potentiation of PH-stimulated Ca transient) in osteoblastic cells. Phenotypic responses to endothelin include stimulation of osteocalcin and osteopontin messages (see herein), inhibition of osteoclast motility and stimulation of prostaglandin-dependent resorption.

- One protein exhibiting channel characteristics was found upregulated. It is
- 20 **aquaporin1** - a water channel protein expressed in many fluid secreting and absorbing tissues such as kidney, brain, heart, eye, inner ear. Its promoter contains glucocorticoid responsive elements and can be activated in response to dexamethasone treatment. Induction of aquaporin-1 expression was detected by subtracted cloning of genes upregulated following cardiopulmonary bypass and
- 25 reperfusion. However, its induction is delayed compared to inflammatory mediators (i.e. ICAM-1, E-selectin, IL-8). The only bone link can be traced in localization of aquaporin molecules in the inner ear, but this localization can be easily explained by the critical dependence of inner ear function on fluid homeostasis. In ear, the protein was found in close association with bone - in most of the cells lining the bony
- 30 labyrinth, and in other non-bony locations.

AHNAK (other names: neuroblast differentiation factor, desmoykin) - a 700 kD protein that was originally identified as differentially repressed (lost) in neuroblastoma cells. Its body is constituted of 128 amino acid repeats. The protein

was initially identified as a nuclear one. However, when it was rediscovered under the name "desmoykin", its subcellular localization was reported as membranal (at the sites of desmosomes). AHNAK-like repeats were found in another protein VAP-1 (vesicle associated protein) - a novel high molecular weight protein found in sea urchin eggs. It is located at peripheral membrane in association with microsomal membrane fraction. Within AHNAK-like repeats of VAP-1 RNA-binding sequences - of RNP1 and of RNP2 types (the same is true for AHNAK). Therefore, it is tempting to speculate, that the general increase in expression of secreted proteins observed in bone tissue in response to mechanical stress might dictate the need in the upregulation of RNA-binding protein localized to a microsomal fraction.

Filamin (non-muscle type), ABP-280, plays a critical role in stabilizing the membrane-cytoskeletal interactions. It is a dimeric actin crosslinking protein that provides the major mode for attaching the cortical F-actin network to membrane glycoproteins. One fillamin molecule is able to crosslink up to 1,000 actin molecules. This ability makes filamin the most potent actin crosslinking agent known today.

Syntrophin 1 is a member of multigene family of intracellular extrinsic membrane proteins found in complex with dystrophin. This particular syntrophin was demonstrated also in complex with nitric oxide (NO) synthase (in muscle tissue). The interaction is likely to be mediated by PDZ domains found in both proteins, but formation of this complex is probably dystrophin-dependent. NO is known to be implicated in the metabolism of bone, especially as a mediator of cytokine effects on remodeling of bone tissue in response to diverse stimuli such as pro-inflammatory cytokines, mechanical stress and sex hormones. Both estrogen and mechanical stress increase NO production by activating constitutive nitric oxide synthase. High concentrations of NO inhibit bone resorption by inhibiting osteoclast formation and by inhibiting the resorptive function of mature osteoclasts, whereas lower NO concentrations potentiate bone resorption and may be essential for normal osteoclast function. On the other hand, growth and differentiation of osteoblasts are also inhibited by high NO concentration.

Thrombospondin 1 - a 450 kD adhesive glycoprotein involved in cellular attachment, spreading, proliferation, and migration. It was originally isolated from plateletes and endothelial cells, but it is also localized in osteoid of undermineralized fetal subperiosteum and in mineralized bone matrix of neonatal/young (growing)

bone. TSP-1 can specifically interact with osteonectin - a 30 kD protein of bones and platelets. This complex formation is Ca-dependent. In osteogenesis imperfecta, levels of osteonectin are reduced, while production of thrombospondin is increased. Expression of thrombospondin is a marker of osteoblast differentiation (together with
5 alkaline phosphatase and alpha-1-collagen). Dexamethasone treatment decreases the levels of thrombospondin expression in cultured osteoblastic cells (glucocorticoids induce osteoporosis). 17-beta estradiol, on the contrary, induces thrombospondin expression. Thrombospondin-1 gene expression is modulated during pericytes differentiation in vitro (pericytes are cells that are embedded within basement
10 membrane of microvessels, believed to participate in angiogenesis, but are able to differentiate into osteogenic phenotype). It is markedly increased during nodule formation and then decreased when mineralization of the nodules has taken place. TSP-1 is excluded from the inner mass of such mineralized nodules.

Several non-thrombospondin genes were found to contain **thrombospondin**
15 **motifs** (cell-binding domain of thrombospondin). One of them also belongs to a metalloproteinase-disintegrin family (identified as an up-regulated gene in the present screen) - **ADAMTS-1**. It was initially cloned as a gene that is selectively expressed in cachectic (in vivo) colon 26 adenocarcinoma subline. It is a putative secreted protein without transmembranal domain. ADAMTS-1 contains six protein modules:
20 pro-, metalloproteinase, disintegrin-like. TSP type 1 motif, spacer, C-terminal TSP motifs.

Another TSP-motifs containing protein is properdin - a plasma glycoprotein which stabilizes the C3nBb enzyme complex of the alternative pathway of the complement system through TSP motifs binding. Interestingly these motifs are also
25 found in terminal complement components C6 - C9.

C3 complement component is produced by osteoblastic and marrow-derived stromal cells in response to vitamin D and regulates differentiation of mononuclear phagocytes into osteoclasts. This effect is bone-specific, since C3 serum, unlike bone, concentrations were unaffected in vitD-deficient mice. In normal mice the C3 protein
30 is located mainly in periosteal regions of calvaria and on the surfaces of bone trabeculae in tibial metaphyses. It is suggested that C3 deposition on mineralized bone surfaces mediates recruitment of mononuclear osteoclasts (unlike multinuclear, express C3 receptor) to this site. In biological fluids, activated C3 in complex with

alpha-2-macroglobulin (whose receptor was found to be upregulated in this screen - this receptor is known to be expressed by bone marrow macrophages, so, probably, osteoclast precursors can be α -2M-receptor positive as well) binds IL-1. It is worth noting, that IL-1 is considered as one of the stimulators of osteoclastogenesis and treatment of ovariectomized mice with its inhibitor significantly decreases the bone loss. Increased osteoclast development after estrogen loss is also mediated by IL-6. Both cytokines expression is upregulated in vivo and in vitro following estrogen deprivation.

It seems now proven that estrogen induces apoptosis of bone-resorbing osteoclasts being applied directly. On the other hand, estrogen induces TGF-beta 1 production by osteoblasts, and anti-TGF-beta antibodies, in turn, can inhibit the estrogen-induced apoptosis of osteoclasts. In this light, finding of **SARP1** upregulation is of special interest. SARPs are a family of secreted apoptosis-related proteins. SARP1 was initially identified as a component of conditioned medium collected from quiescent cells, responsible for apoptosis resistance. SARP2, on the contrary, induces apoptosis sensitization. Structurally SARPs possess a cystein-rich domain (CRD), homologous to CRD of frizzled proteins, but lack the transmembranal domain.

Upregulation of **cytochrom oxidase subunit 1** may be a consequence of mechanical stress or oxidative stress/apoptosis possibly mediated in the system by, for example, increased NO levels.

DEST (ACC#AA177798), after the contige construction turned out to belong to a cDNA coding for **glutamyl-cystein synthetase** - a rate limiting enzyme in glutathione (GSH) synthesis. Its upregulation may be related to the stressed conditions like in the previous case. On the other hand there is one clinical work that correlates GSH reduction (low activity of antioxidant systems) in patients with hypomineralized state of bones.

TGF-beta1 is known as a principal inducer of **connective tissue growth factor** (CTFG, cefl0, fisp12, cyr61, betaIG-M1, beta IG-M2, nov-protoncogene) expression. The latter contains four distinct structural modules, each of them being homologous to distinct domains in other extracellular proteins such as Von Willebrand factor, slit, **trombospondins**, fibrillar collagenes, IGF-binding proteins and mucins. CTGF expression is induced not only by TGF-beta1, but also by **BMP2**

(bone morphogenic factor 2), and during wound repair. In embryogenesis, its expression is found in developing cartilaginous elements, including limbs, ribs, prevertebrae, chondrocranium and craniofacial elements (Meckel's cartilage). Thus, CTGF transcription correlates with differentiation of chondrocytes of both mesodermal and ectodermal origin. In culture, CTGF is expressed in chondrocytes but not in osteoblasts. Possible role in endochondral ossification is suspected because of responsiveness to BMP2. In fibroblasts, CTGF expression causes upregulation of alpha-1-collagen, alpha-5-integrin and fibronectin.

Several enzymes known to participate in steroid synthesis were found transcriptionally elevated in the present system in response to mechanical stress. They include 3-hydroxy-3-methylglutaryl coenzyme A reductase (the first rate limiting enzyme in the chain of cholesterol synthesis from 3 acetyl-CoA molecules), yeast **ERG3 homolog** - sterol-C5-desaturase and yeast **ERG25 homolog** - methyl-sterol-oxidase (both may play a role in formation of cholesterol from lanosterol). It is worth noting that cholesterol is the basis for estrogen and vitamin D3 synthesis. One additional enzyme belonging to fatty acid metabolic pathways that was found upregulated is **stearoyl-CoA desaturase**, that converts the saturated substrate into the D9-deasaturated oleoyl-CoA. Both compounds participate in the synthesis of phospholipids building the cell membrane. Interestingly, estrogens and androsterons are known enhancers of the desaturation reaction.

Alpha-2u microglobulin-related protein (neutrophil gelatinase-associated lipocalin precursor - **NGAL**) belongs to a lipocalin superfamily embracing small extracellular proteins that can bind small hydrophobic molecules (i.e. retinols) and serve ligands to specific extracellular receptors. Many of them were implicated in regulation of cell homeostasis. NGAL was identified as a protein secreted from specific neutrophils' granules upon cell activation and it is identical to a 24p3 protein upregulated in SV-40 induced mitotic reaction. Interestingly, NGAL expression is increased in neu- but not in ras-induced experimental mammary tumors. NGAL can be upregulated by dexamethasone through a responsive promoter element in vitro. In vivo, induction of NGAL in epithelial cells was observed in inflammatory and neoplastic colorectal diseases, but not in normal colon. Among other lipocalins, NGAL is mostly similar to lipocalin-type prostoglandin-D-synthase, responsible for synthesis of prostaglandin D2 from prostaglandin H2. However, NGAL is not

supposed to have the enzymatic activity because of the absence of a specific Cys residue (position 65) which is crucial for prostaglandin-D-synthase function. Structural similarity between two proteins most probably stems from clustered localization of both genes at the same chromosomal locus. Nothing is known about

5 NGAL function in bones.

In addition, two known (RB13-6 antigen and highly charged amino acid sequence ACC# X59131) but without any attributed function and three novel proteins designated 274, 405 and 608 were found to be upregulated in the present model.

RB13-6 is a cell surface 130 kD glycoprotein selectively recognized by

10 monoclonal antibody with the same name. RB13-6 is as a surface antigen of a subset of glial cells highly susceptible to malignant conversion by treatment with a certain carcinogen. This protein is related to the human and murine plasma cell membrane protein PC-1, a nucleotide pyrophosphatase / alkaline phosphodiesterase, and possesses a 5'-nucleotidase activity. However, unlike PC-1, RB13-6 contains an

15 RGD-sequence. The latter is a signature of integrin-interacting proteins.

So called **highly charged amino acid sequence (ACC#X59131)** is a putative protein encoded by anonymous open reading frame of 315 amino acids. It has no significant homology to any protein in the database. Charged amino acids are found in clusters with either Ser and Thr or Ser and Pro residues. Two prominent alpha-helices

20 - one basic and one acidic - are positioned near the C-terminus.

274 (novel gene): In rat calvaria primary cell cultures, expression of this gene was found upregulated approximately 3-fold by mechanical strain. This was detected both by microarray analysis and by Northern hybridization. In rat calvaria this gene is expressed as a single RNA species of approximately 9 Kb. However, expansion of

25 Northern analysis to RNA samples from other rat tissue sources we have found that 274 may probably be alternatively spliced in a tissue specific manner. Alternatively, there is a family of genes closely related to 274 genes that are differently expressed in different tissues. Transcripts of varying length (in general, 9 Kb or a slightly shorter) were found in rat small intestine, skeletal muscle, lung, kidney, eye, brain, colon and

30 testis. The highest expression levels were found in testis, eye and kidney. Complex expression pattern was discovered in bone: two strong transcripts of more than 9 Kb and 4 Kb and three faint transcripts of 1.8 Kb, 0.5 Kb and 0.3 Kb. Interestingly, when human derived lymphoid cell line NB4 was hybridized to the same probe, these three

faint transcripts appeared strong, while bone-specific two strong transcripts were not evident. This, probably, indicates that the origin of the three short transcripts is in some lymphoid precursors present in small amount in the bone marrow.

cDNA library was prepared from RNA extracted from calvaria cells after
5 mechanical stimulation. 5291 bp 274-specific RACE product was synthesized and
sequenced (see Figs 2A-14). Comparison to public databases revealed that 274 is a rat
homologue (98% identity on the level of amino acids) of anonymous human cDNA
KIAA0462. This sequence is 7150 bp long and contains open reading frame of 6900
bp capable of coding for 2300 amino acid protein. The frame is still open from the 5',
10 indicating the lack of the N-terminal sequences. The open reading frame extends to
the 5' direction for additional 900 bp compared to the human KIAA0462 sequence,
but still does not reach the beginning of the protein. On the basis of human sequence
information the inventors were able to synthesize a 6.8 Kb long human specific
RACE contig. The putative KIAA0462 protein has no direct analogs in the database.
15 It distantly (26%) resembles the *C.elegans* hypothetical protein (AF003140) that was,
in turn, defined as having weak similarity to the drosophila hyperplastic disc protein.
No known protein functional domains were identified either. A stretch of 24
hydrophobic amino acids between positions 165 and 188 of the KIAA0462 putative
protein hints on its potential transmembranal location.

20 In situ hybridization analysis (discussed in more detail in further Examples,
infra): in normal rat bones and bones obtained from ovariectomized (osteoporotic)
rats gave preliminary results indicating that gene 274 is expressed in long bones of
normal rats in lining cells covering the inner surface of compact bone and in bone
marrow. In normal trabecular bone specific signal was detected in bone marrow.
25 Osteoporotic bones were 274 – negative or displayed extremely reduced signal.

CMF274 appears to encode a huge protein. This gene seems to be bone
specific. In the bone expression is in many compartments. Expressed in osteoblasts
but not in lining cells. This gene seems different from the other two in that it is
expressed in more mature cells.

30 With respect to CMF 274, mention is made of Xu et al, "Retinal Targets for
Calmodulin Include Proteins Implicated in Synaptic Transmission," J Biol Chem
273(47):31297-307 (1998). Xu relates to what may be a homolog for CMF274 in
drosophila, named "calossin". Xu et al. may provide homology to mouse ESTs and

that the protein is highly conserved during evolution. The mouse ESTs may be from the mouse homolog of CMF274. However, in Xu et al., the mouse gene was not characterized, and Xu et al. do not provide any relation to bones.

CMF274 contains some interesting domains including calmodulin binding
5 domain and two zinc finger domains. The first implies capability to bind the important Calcium sensor calmodulin, and the second implies DNA binding capabilities. This combination support the proposition that CMF274 is involved in a central aspect of bone biology: sensing amount of calcium and translating it into nuclear signals that change the expression of downstream genes. It is important to note that one of the
10 zinc-finger binding domains (CRD1) "is most similar to the zinc-finger family defined by Requiem, a protein required for apoptosis" (Xu et al).

405 (novel gene): In rat calvaria primary cell cultures, expression of this gene was found to be downregulated in response to mechanical stimulation as detected both by microarray analysis and by Northern blots. A single 9 Kb transcript was detected
15 in this tissue. However, being hybridized to rat tissue blot, the same probe had detected a major 5 Kb transcript ubiquitously expressed in bone, brain, colon, small intestine, testis, ovary, uterus, heart, kidney, liver, stomach, thymus, spleen, bladder, adipose tissue and mammary gland.

Partial 405 rat cDNA clone was isolated from rat calvaria cDNA library by
20 RACE technique and sequenced. It contains 3684 bp, 3000 of them constituting an open reading frame closed from the 3' end (see Figs 2A-14). Comparison to public databases revealed that gene 405 is a rat homologue of human anonymous cDNA sequences KIAA0183 and AF055017. Interestingly, the latter cDNA lacks a 294 bp (98 amino acids) fragment corresponding to positions 2384 - 2483 of KIAA0183
25 putative amino acid sequence. Rat 405 homologue contains this region.

Thus, there is an indication that gene 405 is subjected to alternative splicing. KIAA0183 cDNA clone was obtained from Genbank and used as a probe for hybridization with human tissue RNA blot. As in the case of rat tissues, a 5 Kb transcript was detected. However, in humans its expression was not as uniformly
30 distributed among different tissues as in rats. The highest levels were detected in early embryo, and in testis, placenta, ovary, tongue, intestine in adults. The only RNA of human origin where a faint 9 Kb transcript (together with the major 5 Kb one) could be seen was RNA from K562 early myeloid precursor cell line.

KIAA0183 cDNA clone represents a 1062 amino acid open reading frame, lacking the N-terminus. The available sequence has no transmembrane domain. On the other hand, four structural subdomains can be easily identified: N-terminal, highly charged alpha-helical region, Ser-Pro rich spacer domain, C-terminal highly charged alpha-helical region, and a tail region, rich in Ser, Pro, Gly, and Arg - amino acids, known to be clustered in this composition in the RNA-binding protein regions. A middle spacer region contains an RGD motif known to serve as a receptor to integrins. No significant homology to any known protein was found. Among those ones that displayed distant fragment homology, are FUS/TLS RNA binding protein (nuclear export) and various types of collagens.

Thus, as CMF405 the full sequence of the human cDNA is also presented. The pattern of expression in bone suggests involvement in osteoblast and chondrocyte differentiation. The presence of an RGD motif in this protein suggests involvement in response to integrins. Expression in few other tissues may suggest a broader function.

608 (novel gene): In rat calvaria primary cell cultures, expression of this gene was found upregulated approximately 3-fold by mechanical strain. This was detected both by microarray analysis and by Northern hybridization. In rat calvaria this gene is expressed as a single RNA species of approximately 9 Kb. Hybridization signal was not detected in any other rat RNA from different tissue sources, including testis, colon, intestine, kidney, stomach, thymus, lung, uterus, heart, brain, liver, eye, and lymphnode.

Partial 608 rat cDNA clone was isolated from rat calvaria cDNA library by RACE technique and sequenced. RACE contig is 4007 bp long and contains a 3356 bp open reading frame closed from the 3'. Comparison to public databases revealed no sequence homologues. There are several human EST clones, similar 608 cDNA. The primary structure of the putative protein enables to attribute it to Ig superfamily.

By *in situ* hybridization (discussed in more details in Examples *infra*), expression of gene 608 was found in bone marrow from normal trabecular bones, but not of osteoporotic ones.

As to CMF608, the inventors found that it encodes a big protein that is most probably a part of the extra-cellular matrix. The gene may be actively involved in supporting osteoblast differentiation. Another option is that it marks regions were

remodeling takes place. Such an hypothesis is also compatible with a role in directing osteoclast action and thus it may be a target for inhibition by small molecules.

In normal bone formation, activation of osteoblasts leads to secretion of various factors that attract osteoclast precursors or mature osteoclasts to the sites of bone formation to initiate the process of bone resorption. In normal bone formation both functions are balanced. Imbalance to any side causes either osteopetrosis (osteoblast function overwhelms) or osteoporosis (osteoclast function overwhelms).

Among known osteoblast activators - mechanical force stimulation - is actually applied in the present model. As proof of principle, increased expression of several genes known to respond to mechanical stress by transcriptional upregulation were found. They include tenascin, endothelin and possibly thrombospondin. Upregulation of water channel encoding message is likely related to this mechanism too.

Among genes whose expression is found upregulated are those known to be expressed by activated osteoblasts (i.e. complement C3) or those whose upregulation may be logically connected:

1) Mechanical stress activates constitutive NO-synthase (NO plays an important role in bone building). In muscle, this enzyme is found in complex with syntrophin 1. The latter is found upregulated in our screen. Therefore, it can participate in NO-synthase activation in bone, too.

2) Some proteins secreted by osteoblasts participate osteoclast attraction through the RGD-mediated binding to integrin receptor highly expressed by osteoclasts, e.g., osteopontin. While this specific protein was not identified in the present screen, this is of no moment as other RGD-containing proteins were identified in the present screen, including the DEST405 and RB13-6.

3) Additional protein complex that attracts osteoclasts is the complex of C3 complement. Such complexes are known to be stabilized in plasma by a thrombospondin motifs containing glycoprotein - properdin. Another thrombospondin motifs-containing protein without any known function - ADAMTS-1 is found upregulated. It can participate in stabilization of C3 complexes in bone.

Several lines of evidence indicate that mechanical stress causes generation of both apoptotic (NO, cytochrom oxidase subunit 1) and antiapoptotic signalling

(SARP1, glutamyl-cystein synthetase). That may be important for keeping the balance between osteoblast and osteoclast proliferation, differentiation and death.

Interestingly, the inventors found that in response to mechanical force, several enzymes that regulate the chain of chemical reactions potentially leading steroid synthesis are upregulated. In bone, these steroids may have the estrogen-like function that is reflected by at least two observations: first - upregulation of stearoyl-CoA-desaturase (elevation of this enzyme activity is known as estrogen-dependent); and, second - upregulation of connective tissue growth factor that is mainly induced in cells by TGF-beta -1, that, in turn, is known to be induced in osteoblasts by estrogen. Such a link may explain a common anti-osteoporotic action of estrogens and mechanical force. It is worth noting, that estrogen induces osteoclasts apoptosis both directly (applied to cultured osteoclasts) and in TGF-beta-1-dependent manner.

Genes that are implicated in osteoporosis, osteoporosis prevention, treatment, or control, or in study or investigation to advance knowledge of osteoporosis, its prevention, treatment or control and/or in bone growth/formation study or investigation and/or for addressing maladies, conditions, symptoms, and the like, associated with bone growth/formation, include three novel genes DEST's 274, 405, 608, as well as RG13-6 (as RGD-containing protein), metalloproteinase ADAMTS-1, and proteins of SARP family (secreted apoptosis related proteins) including as potential modifiers of programmed cell death in bone formation.

Furthermore, as mentioned herein, this Example can also be performed without imparting mechanical stress to the cells; for instance, in reduced or zero gravity conditions, to develop a model with the lack of mechanical stress; and, the invention comprehends such a model and genes thereby identified.

Example/Result 3: CMF608 GENE EXPRESSION BY *IN SITU* HYBRIDIZATION

Pattern of expression of CMF608 gene was studied by in situ hybridization on sections of bones from ovariectomized and sham-operated rats. Female Wistar rats weighting 300-350 g were subjected to ovariectomy under general anesthesia. Control rats were operated in the same way but ovaries were not excised - sham operation.

Three weeks after operation rats were sacrificed and tibia were excised together with the knee joint. Bones were fixed for three days in 4% paraformaldehyde and then decalcified for four days in solution containing 5% formic acid and 10%

formalin. Decalcified bones were postfixed in 10% formalin for three days and embedded into paraffin.

To study the pattern of expression of CMF608 gene in developing bone the model of ectopic bone formation was employed. Rat bone marrow cells were seeded
5 into cylinders of demineralized bone matrix prepared from rat tibiae. Cylinders were implanted subcutaneously into adult rats. After three weeks rats were sacrificed and implants were decalcified and embedded into paraffin as described above for tibial bones.

The 6 μ m sections were prepared and subjected to *in situ* hybridization
10 procedure. After hybridization sections were dipped into nuclear track emulsion and exposed for three weeks at 4°C. Autoradiographs were developed, stained with hematoxylin-eosin and studied under microscope using brightfield and darkfield illumination.

For the further assessment of cell and tissue specificity of CMF608 gene
15 expression *in situ* hybridization study was performed on sections of multitissue block containing multiple samples of adult rat tissues. Developmental pattern of CMF608 expression was studied on sagittal sections of mouse embryos of 12.5, 14.5 and 16.5 days postconception (dpc) stages.

Microscopic study of hybridized sections of long bones revealed a peculiar
20 pattern of CMF608 probe hybridization. The hybridization signal can be seen mainly in fibroblast-like cells found in several locations throughout the sections. Prominent accumulations of these cells can be seen in the area of periosteal modeling in metaphysis, and also in regions of active remodelling of compact bone in diaphysis: at the boundary between bone marrow and endosteal osteoblasts and in periosteum, also
25 in close contact with osteoblasts. Perivascular connective tissue filling Volkmann's canals in compact bone in diaphysis and epiphysis also contains expressing cells. No hybridization is found within cancellous bone and in bone marrow. This pattern of hybridization suggests that cells showing expression of CMF608 are associated with areas of remodelling of preexisting bone and are not involved in primary
30 endochondral ossification.

At the level of growth plate expressing cells can be seen in perichondral fibrous ring of LaCroix. Some investigators regard this fibrous tissue as the aggregation of residual mesenchymal cells able to differentiate into both osteoblasts

and chondrocytes. In this respect it is noteworthy that single cells expressing CMF608 can be seen in epiphyseal cartilage. These expressing cells are rounded cells within the lateral segment of epiphysis (sometimes in close vicinity to the ring of LaCroix) and flattened cells covering the articulate surface. Most of cells in articulate cartilage and all chondrocytes of growth plate do not show expression of CMF608. Ovariectomy did not result in change of the intensity and pattern of CMF608 expression in bone tissue.

In sections of ectopic bones hybridization signal for CMF608 can be seen in some fibroblast-like cell either scattered within unmineralized connective tissue matrix or concentrated at the boundary between this tissue and osteoblasts of immature bone.

Pattern of expression of CMF608 gene revealed by in situ hybridization in bone and cartilage allows to speculate that its expression marks some skeletal tissue elements able to differentiate into two skeletal cell types - osteoblasts and chondrocytes. The terminal differentiation of these cells appears to be accompanied by down-regulation of CMF608 expression. The latter suggestion is supported by peculiar temporal pattern of CMF608 expression in primary cultures of osteogenic cells isolated from calvaria bones of rat fetuses. In these cultures expression was revealed by in situ hybridization in vast majority of cells after one and two weeks of incubation in vitro. Three and four weeks old cultures showing signs of ossification contain no expressing cells. Significantly, no hybridization signal was found on sections of multitissue block hybridized to CMF608 probe suggesting high specificity of this gene expression for the skeletal tissue in adult organism.

In situ hybridization study of embryonic sections demonstrated that at 12.5 dpc weak hybridization signal can be discerned in some mesenchymal cells in several locations throughout the embryonic body. The most prominent signal is found in the head: in loose mesenchymal tissue surrounding the olfactory epithelium and underlying the surface epithelium of nose tip. Other mesenchymal cells in the head also show hybridization signal: in non-cartilagenous part of basisphenoid bone primordium and in mesenchyme surrounding the dental laminae (tooth primordia) in the mandible.

In the trunk expression can be detected in less developed vertebrae primordia in thoraco-lumbar region. Hybridization signal here marks the condensed portion of

sclerotomes. Another area showing hybridization signal in the trunk is comprised by thin layer of mesenchymal cells in the anterior part of thoracic body wall.

At later stages of development -14.5 and 16.5 dpc probe CMF608 gave no hybridization signal. Thus, it appears that during embryonic development CMF608 gene is transiently expressed by at least some mesenchymal and skeleton-forming cells. This expression is down-regulated at later stages of development. More detailed study of late embryonic and postnatal stages of development will reveal the timing of appearance of CMF608 expressing cells in bone tissue.

Example/Result 4: CMF405 GENE EXPRESSION BY *IN SITU*

10 HYBRIDIZATION

Pattern of expression of CMF405 gene was studied by in situ hybridization on sections of bones from ovariectomized and sham-operated rats. Female Wistar rats weighting 300-350 g were subjected to ovariectomy under general anesthesia. Control rats were operated in the same way but ovaries were not excised - sham operation.

15 Three weeks after operation rats were sacrificed and tibia were excised together with the knee joint. Bones were fixed for three days in 4% paraformaldehyde and then decalcified for four days in solution containing 5% formic acid and 10% formalin. Decalcified bones were postfixed in 10% formalin for three days and embedded into paraffin.

20 To study the pattern of expression of CMF405 gene in developing bone the model of ectopic bone formation was employed. Rat bone marrow cells were seeded into cylinders of demineralized bone matrix prepared from rat tibiae. Cylinders were implanted subcutaneously into adult rats. After three weeks rats were sacrificed and implants were decalcified and embedded into paraffin as described above for tibial bones.

25 The 6 μ m sections were prepared and subjected to in situ hybridization procedure. After hybridization sections were dipped into nuclear track emulsion and exposed for three weeks at 4°C. Autoradiographs were developed, stained with hematoxylin-eosin and studied under microscope using brightfield and darkfield illumination.

30 For the further assessment of cell and tissue specificity of CMF405 gene expression in situ hybridization study was performed on sections of multitissue block containing multiple samples of adult rat tissues. Developmental pattern of CMF405

expression was studied on sagittal sections of mouse embryos of 12.5, 14.5 and 16.5 days postconception (dpc) stages.

Bones: Hybridization signal for CMF405 gene is widely spread throughout different cell types on sections of long bones from sham-operated animals: cartilage,
5 bone marrow and bone.

In the growth plate hybridization signal is concentrated in the transition zone from proliferating to hypertrophic cartilage so that most advanced proliferating chondrocytes and youngest hypertrophic chondrocytes display expression. Both young proliferating chondrocytes and most of mature hypertrophic chondrocytes do
10 not show hybridization signal. Chondrocytes of articulate cartilage show no hybridization signal.

Some (but not all) hematopoietic cells within the bone marrow show clear hybridization signal. Poor morphology of decalcified section stained with hematoxylin-eosin does not allow identification of expressing cell types.

15 Within the bone tissue hybridization signal can be seen in osteoblasts localized in primary spongiosa and secondary spongiosa in metaphysis (cancellous bone). Osteoblasts covering the surface of marrow cavity and Volkmann's canals in diaphyseal (compact) bone also display hybridization signal. Flat bone lining cells and osteocytes are not expressed in any part of the bone.

20 Ovariectomy did not result in change of the intensity and pattern of CMF405 expression. In ectopic bone hybridization signal concentrates mainly in osteoblasts of immature bone. This signal is weak or absent from osteoblasts embedded into bone matrix.

The pattern of hybridization of CMF405 gene in adult skeletal tissues suggests
25 that its expression is characteristic for osteogenic and chondrogenic cells at intermediate stage of their differentiation preceding intensive matrix calcification.

Tissue expression: The CMF405 probe was hybridized to multitissue block sections. The hybridization signal can be seen mainly in epithelial cells in many organs and tissues suggesting the wide expression of this gene in adult tissues.

30 The hybridization signal of varying intensity can be seen in epithelial lining of the digestive system. Weak hybridization signal can be seen in basal cells of stratified squamous epithelium of esophagus. Weak signal is displayed by surface epithelium of fundic stomach. In pyloric stomach strong hybridization signal is displayed by cells

lining mucosal pits and weaker signal - by surface epithelium. In thin intestine expressing cells are localized in crypts and glands while villous epithelium shows no hybridization signal. Similar pattern is observed in colonic epithelium: weak hybridization signal can be seen only in crypts and not in villi. This pattern of hybridization throughout different parts of the alimentary canal allows to suggest that expression of CMF405 in digestive system appears to be confined mainly to actively proliferating epithelial cells and transition of epithelial cells into non-proliferative compartment (like suprabasal layers of esophagus or villous epithelium of intestine) is accompanied by down-regulation of CMF405 expression.

10 In urogenital system weak and diffuse signal can be seen in medullar part of kidney. The weak hybridization signal is displayed by transitional epithelium of kidney calyx, ureter and bladder. Hybridization signal is seen also in basal cells within seminiferous tubules. Low resolution of microautoradiographs does not allow unequivocal identification of expressing cells as spermatogonia or Sertoli cells.

15 All epidermal layers of skin show hybridization signal. Strongly expressing cells are localized also in all layers of the hair follicle.

Strong and uniform hybridization signal can be seen on sections of lymphoid organs: thymus, spleen and lymph nodes.

20 Positive hybridization was obtained also on eye sections: strong signal is displayed by corneal epithelium. Retina shows weaker hybridization signal throughout all layers excluding ganglion cell layer.

No hybridization signal can be seen in brain.

25 *In situ* hybridization study of sections of 12.5, 14.5 and 16.5 dpc embryos revealed strong and practically uniform hybridization signal throughout bodies of 12.5 and 14.5 dpc embryos. This suggests that all cell types at these stages of development express CMF405 gene. By 16.5 dpc stage expression appears to decline in some cells so that pattern of expression approaches to that in adult tissue although some structures showing no expression in adults display hybridization signal.

Example/Result 5: EXPRESSION OF CMF274 GENE BY *IN SITU* HYBRIDIZATION

Hybridization of CMF274 probe to the sections of knee joint demonstrated wide expression throughout the bone, cartilage and bone marrow tissues.

- 5 Hybridization signal can be seen in hematopoietic bone marrow cells. Accumulation of signal in cells of eosinophilic lineage is clear. Poor morphology of decalcified sections does not allow identification of other expressing cell types although it is apparent that not all the myeloid elements are expressing.

- 10 Chondrocytes display weak hybridization signal throughout all zones of growth plate. No expression is detected in epiphyseal articulate cartilage.

- Within the bone tissue hybridization signal can be seen in osteoblasts localized in primary spongiosa and secondary spongiosa in methaphysis (cancellous bone). Periosteal and endosteal osteoblasts and osteoblasts of Volkmann's canals in diaphyseal (compact) bone also display hybridization signal. Flat lining cells and
15 osteocytes are not expressing in any part of the bone. Ovariectomy did not result in change of the intensity and pattern of CMF274 expression in bone tissue.

The CMF274 probe was hybridized to multitissue block sections and to sections of 12.5, 14.5 and 16.5 dpc mouse embryos. No hybridization signal was found on these sections.

20 **Example/Result 6: CMF2-45 (SARP) GENE EXPRESSION BY *IN SITU* HYBRIDIZATION**

Hybridization signal for CMF2-45 (SARP) gene is found in different cell types on sections of long bones from sham-operated animals: cartilage, bone marrow and bone. In all expressing cell types the level of hybridization signal is rather low.

- 25 In growth plate hybridization signal marks proliferating chondrocytes while hypertrophic chondrocytes show little or no signal. Chondrocytes of articulate cartilage in epiphysis do not show hybridization signal.

- Hybridization signal in bone tissue proper marks osteoblasts located in all compartments of cancellous and compact bone: primary and secondary spongiosa, periosteum, endosteum and Volmann's canals. Bone lining cells and osteocytes show
30 no hybridization signal.

Some (but not all) hematopoietic cells within the bone marrow show clear hybridization signal. Poor morphology of decalcified sections stained with hematoxylin-eosin does not allow identification of expressing cell types.

In ectopic bone hybridization signal can be seen in osteoblasts of immature bone. Beside of osteoblasts single fibroblast-like cells scattered throughout the connective tissue also show hybridization signal. Some of these expressing fibroblast-like cells can be seen in close contact with osteoblasts.

**Example/Result 7: CMF2-224 (Rb13-6) EXPRESSION
BY IN SITU HYBRIDIZATION**

In situ hybridization study of RB13-6 gene (GenBank Accession No.: Z47987) revealed expression of this gene in osteoblasts located in different compartments of long bone: in primary and secondary spongiosa of cancellous bone in metaphysis and within endosteum and Volkmann's canals of compact bone in diaphysis. Weak hybridization signal can be seen also in myeloid cells of bone marrow. Osteoblasts of immature bone developing within ectopic bone implants also display hybridization signal. Significantly, no hybridization signal was revealed on sections of long bones of ovariectomised rats and also on sections of ectopic bones implanted into ovariectomised rats. This result suggests that expression of RB13-6 in osteoblasts and bone marrow cells is estrogen-dependent. Further study will be needed to clarify the involvement of RB13-6 gene product into regulation of osteoblast function and development of osteoporosis.

Additional *in situ* hybridization study on sections of multitissue block demonstrated expression of RB13-6 gene in distinct epithelial cell types and in lymphoid tissue. Expressing cells can be seen in epithelial lining of bronchi, villous (i.e. mature and non-proliferating) epithelium of thin intestine, in luminal and glandular epithelia of uterus, acinar and ductal epithelia of salivary glands. Very weak hybridization signal suggesting low level of expression was found in liver and in kidney. Liver expression appears to be uniform throughout hepatocytes. In kidney single expressing cells can be seen in thick ascending part of Henle's loop.

Within lymphoid tissue strongly expressing lymphocytes are concentrated in medullar zone of lymph nodes. Few expressing cells can be seen in spleen: in the perifollicular zone (the bordering area between the red and white pulp) and in perivascular aggregations of lymphocytes. No expressing cells were found in thymus.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited by particular details set forth in the above description as many
5 apparent variations thereof are possible without departing from the spirit or scope thereof.

References

- Consensus Development Conference: Prophylaxis and Treatment of Osteoporosis: *Am J Med* 94:646-650, 1993
- 5 WHO Study Group: In *Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis*. WHO Technical Report Series: 843, 1994
- Department of Health *Health and Personal Social Services Statistics for England*,
 10 1991 Edition. HMSO, London, 1991, pp 10-11
- Welsh Office *Health and Personal Social Services for Wales* HMSO. London, 1991, pp 2-3
- 15 Kanis JA, McCloskey EV: Epidemiology of vertebral osteoporosis. *Bone* 13:S1-S10, 1992
- Kanis JA, Pitt FA: Epidemiology of osteoporosis. *Bone* 13:S7-S15, 1992
- 20 Cummings SR et al: Epidemiology of osteoporosis and osteoporotic fractures. *Epidemiol Rev* 7:178-207, 1985
- Department of Health and Social Security Office of Population Censuses and Surveys *Cancer Statistics: Registrations*. Series MBT, 20, HMSO, London, 1987
- 25 Riggs BL, Melton LJ: Involutional osteoporosis. *N Engl J Med* 314:1676-1686, 1986
- Melton LJ, Eddy DM, Johnston CC: Screening for osteoporosis. *Ann Intern Med* 112:516-528, 1990
- 30 Department of Health and Social Security Office of Population Censuses and Surveys *Hospital In-Patient Enquiry Summary Tables*. Series MB4, 26, HMSO, London, 1985
- Nordin BEC, Need AG: How can we prevent osteoporosis? In *Osteoporosis 1987* (Christiansen C, Riis BJ, eds.). Osteopress ApS, Copenhagen, 1987, pp 1204-1210
- 35 United Nations *Interpolated National Populations by Age and Sex: 1950-2025 (1992 Revision)*. United Nations Population Division, United Nations, New York, 1992
- 40 Cooper C, Campion G, Melton LJ: Hip fractures in the elderly: A world-wide projection. *Osteoporosis Int* 2:285-289, 1992
- Parfitt AM: Quantum concept of bone remodeling and turnover: Implications for the pathogenesis of osteoporosis. *Calcif Tissue Int* 28: 1-5, 1979
- 45 Parfitt AM: Bone remodeling in the pathogenesis of osteoporosis. *Res Staff Physician*, December:60-72, 1981

- Parfitt AM: Bone remodeling: Relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In *Osteoporosis: Etiology, Diagnosis, and Management* (Riggs BL, Melton LJ, eds.). Raven Press, New York, 1988, pp 74-93
- 5 Kanis JA *Pathophysiology and Treatment of Paget's Disease of Bone*. Martin Dunitz, London, 1991, pp 12-40
- 10 Smith DM et al: Genetic factors in determining bone mass. *J Clin Invest* 52:2800-2808, 1973
- Pocock NA et al: Genetic determinants of bone mass in adults: A twin study. *J Clin Invest* 80:706-710, 1987
- 15 Matkovic V et al: Factors that influence peak bone mass formation: A study of calcium balance and the inheritance of bone mass in adolescent females. *Am J Clin Nutr* 52:878-888. 1990
- 20 Cohn SH et al: Comparative skeletal mass and radial bone mineral content in black and white women. *Metabolism* 26:171-178, 1977
- Selby PL: Endocrinology and osteoporosis. In *HRT and Osteoporosis* (Drife JO, Studd JWW, eds.). Springer Verlag, London, 1990, pp 105-114
- 25 Stevenson JC et al: Exercise and the skeleton. In *Osteoporosis 1990* (Smith R, ed.). Royal College of Physicians of London, London, 1990, pp 119-124
- Griffin J: *Osteoporosis and the Risk of Fracture*. Office of Health Economics, London, 1990, p 48
- 30 Mundy GR *Calcium Homeostasis: Hypercalcemia and Hypocalcemia*, 2nd ed. Martin Dunitz, London, 1990, pp 1, 32, 51-52, 179, 200
- 35 Christiansen C, Riis B *The Silent Epidemic: Postmenopausal Osteoporosis*. Christiansen and Riis, Denmark, 1990, pp 23, 66-67, 70-73
- Christiansen C, Rodbro P, Tjellesen L: Serum alkaline phosphatase during hormone treatment in early postmenopausal women: A model for establishing optimal prophylaxis and treatment in postmenopausal osteoporosis. *Acta Med Scand* 216:11-17, 1984
- 40 Gallagher JC et al: Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients: Effect of age and dietary calcium. *J Clin Invest* 64:729-736, 1979
- 45 Heaney RP: Nutritional factors in bone health. In *Osteoporosis: Etiology, Diagnosis, and Management* (Riggs BL, Melton LJ, eds.). Raven Press, New York, 1988, pp 359-372

- Heaney RP: Effect of calcium on skeletal development, bone loss, and risk of fractures. *Am J Med* 91 (suppl 5B):5B-23S-5B-29S, 1991
- 5 Delmas PD et al: Increase in serum bone gamma-carboxyglutamic acid protein with aging in women: Implications for the mechanism of age-related bone loss. *J Clin Invest* 71:1316-1321, 1983
- Drinkwater BL et al: Bone mineral content of amenorrheic and eumenorrheic athletes. *N Engl J Med* 311:277-281, 1984
- 10 Silverman SL: The clinical consequences of vertebral compression fracture. *Bone* 13:S27-S31, 1992
- Adami S et al: The radiological assessment of vertebral osteoporosis. *Bone* 15 13:S33-S36, 1992
- Riggs BL: Practical management of the patient with osteoporosis. In *Osteoporosis: Etiology, Diagnosis, and Management* (Riggs BL, Melton LJ, eds.). Raven Press, New York, 1988, pp 481-490
- 20 Kanis JA et al: Screening techniques in the evaluation of osteoporosis. In *HRT and Osteoporosis* (Drife JO, Studd JWW, eds.). Springer Verlag, London, 1990, pp 135-147
- 25 Genant HK, Faulkner KG, Gluer C-C: Measurement of bone mineral density: Current status. *Am J Med (suppl 5B)* 91:5B-49S-5B-53S, 1991
- Sartoris DJ, Resnick D: Digital radiography may spark renewal of bone densitometry. *Diagnostic Imaging*, January:145-150, 1988
- 30 Fogelman I, Rodin A: The measurement of bone density. In *HRT and Osteoporosis* (Drife JO, Studd JWW, eds.). Springer Verlag, London, 1990, pp 119-133
- Mazess RB et al: Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 51:1106-1112, 1990
- 35 Johnston CC et al: Clinical indications for bone mass measurements. *J Bone Min Res* 4 (suppl 2): 1-29, 1989
- 40 Delmas PD: Clinical use of biochemical markers of bone remodeling in osteoporosis. In *Osteoporosis 1990* (Christiansen C, Overgaard K, eds.). Osteopress ApS, Copenhagen, 1990, pp 450-458
- Uebelhart D et al: Urinary excretion of pyridinium crosslinks: A new marker of bone resorption in metabolic bone disease. *Bone Mineral* 8:87-96, 1990
- 45 Consensus Development Conference: Prophylaxis and Treatment of Osteoporosis: *Am J Med* 90:107-110, 1991

- Delmas PD: Biochemical markers for the assessment of bone turnover. In *Osteoporosis: Etiology, Diagnosis, and Management*. Rev. ed. (Riggs BL, Melton LJ, eds.). Raven Press, New York, 1995, pp 319-333
- 5 Uebelhart D et al: Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium cross-links. *J Clin Endocrinol Metab* 72:367-373, 1991
- 10 Christiansen C, Riis BJ, Rodbro P: Screening procedure for women at risk of developing postmenopausal osteoporosis. *Osteoporosis Int* 1:35-40, 1990
- Sinaki M: Exercise and physical therapy. In *Osteoporosis: Etiology, Diagnosis, and Management* (Riggs BL, Melton LJ, eds.). Raven Press, New York, 1988, pp 457-479
- 15 Davee AM et al: Exercise patterns and trabecular bone density in college women. *J Bone Min Res* 5:245-250, 1990
- Gleeson PB et al: Effects of weight lifting on bone mineral density in premenopausal women. *J Bone Min Res* 5:153-158, 1990
- 20 Riggs BL, Melton LJ: The prevention and treatment of osteoporosis. *N Engl J Med* 327:620-627, 1992
- 25 Riggs BL: Treatment of osteoporosis with sodium fluoride or parathyroid hormone. *Am J Med* (suppl 5B) 91:5B-37S-5B-41S, 1991
- Kanis JA et al: Osteoporosis: Causes and therapeutic implications. In *Osteoporosis 1990* (Smith R, ed.). Royal College of Physicians of London, London, 1990, pp 45-56
- 30 Rogers HJ, Spector AG, Trousseau JR: In *A Textbook of Clinical Pharmacology*. Hodder and Stoughton, London, 1981, p 583
- Eriksen EF et al: Evidence of estrogen receptors in normal human osteoblast-like cells. *Science* 241 :84-86, 1988
- 35 Lindsay R, Cosman F: Estradiol in prevention of osteoporosis. In *Osteoporosis 1990* (Christiansen C, Overgaard K, eds.). Osteopress ApS, Copenhagen, 1990, pp 2070-2075
- 40 Lindsay R: Estrogens, bone mass, and osteoporotic fracture. *Am J Med* 91 (suppl 5B):5B10S-5B-13S, 1991
- Lindsay R, Tohme JF: Estrogen treatment of patients with established postmenopausal osteoporosis. *Obstet Gynecol* 76:290-295, 1990
- 45 Christiansen C, Riis BJ: 17 β -estradiol and continuous norethisterone: A unique treatment for established osteoporosis in elderly women. *J Clin Endocrinol Metab* 71:836-841, 1990

- Lufkin EG et al: Treatment of postmenopausal osteoporosis with transdermal estrogen. *Ann intern Med* 117:1-9, 1992
- 5 Segre G, Bruni G, Dal Pra P: Calcitonin pharmacokinetics. In *Calcitonin* (Pecile A, ed.). Elsevier, New York, 1985, pp 99-107
- Nicholson GC et al: Abundant calcitonin receptors in isolated rat osteoclasts. *J Clin Invest* 78:355-360, 1986
- 10 Baron R, Vignery A: Behaviour of osteoclasts during a rapid change in their number induced by high doses of parathyroid hormone or calcitonin in intact rats. *Metab Bone Dis Rel Res* 2:339-343, 1981
- Overgaard K et al: Effect of calcitonin given intranasally on early postmenopausal bone loss. *Br Med J* 299:477-479, 1989
- 15 Rasmussen H et al: Effect of combined therapy with phosphate and calcitonin on bone volume in osteoporosis. *Metab Bone Dis Rel Res* 2:107-111, 1980
- 20 Gruber HE et al: Long-term calcitonin therapy in postmenopausal osteoporosis. *Metabolism* 33:295-303, 1984
- Christiansen C et al: Intranasal calcitonin in the treatment of postmenopausal osteoporosis. In *Osteoporosis 1987* (Christiansen C, Riis BJ, eds.). Osteopress ApS, Copenhagen, 1987, pp 853-856
- 25 Overgaard K et al: Nasal calcitonin for treatment of established osteoporosis. *Clin Endocrinol* 30: 435-442, 1989
- 30 Overgaard K et al: Discontinuous calcitonin treatment of established osteoporosis: Effects of withdrawal of treatment. *Am J Med* 89:1-6, 1990
- Fleisch H: Bisphosphonates: Pharmacology and use in the treatment of tumour-induced hypercalcemic and metastatic bone disease. *Drugs* 42:919-944, 1991
- 35 Watts NB et al: Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. *N Engl J Med* 323:73-79, 1990
- Filipponi P et al: Cyclical clodronate is effective in preventing postmenopausal bone loss: A comparative study with transcutaneous hormone replacement therapy. *J Bone Min Res* 10:697-703, 1995
- 40 Chesnut CH et al: Alendronate treatment of the postmenopausal osteoporotic woman: Effect of multiple dosages on bone mass and bone remodeling. *Am J Med* 99: 144-152, 1995
- 45 Gamero P et al: Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endocrinol Metab* 79(6):1693-1700, 1994

- Francis RM: The calcium controversy. In *Osteoporosis 1990* (Smith R, ed.). Royal College of Physicians of London, London, 1990, pp 125-133
- 5 Johnston CC et al: Calcium supplementation and increases in bone mineral density in children. *N Engl J Med* 327:82-87, 1992
- Baran D et al: Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: A three-year prospective study. *J Clin Endocrinol Metab* 70:264-270, 1989
- 10 Dawson-Hughes B et al: A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med* 323:878-883, 1990
- 15 Chapuy MC et al: Vitamin D3 and calcium to prevent hip fractures in elderly women. *N Engl J Med* 327:1637-1642, 1992
- Hassager C et al: Changes in soft tissue body composition and plasma lipid metabolism during nandrolone decanoate therapy in postmenopausal osteoporotic women. *Metabolism* 38:238-242, 1989
- 20 Johansen JS et al: Treatment of postmenopausal osteoporosis: Is the anabolic steroid nandrolone decanoate a candidate? *Bone Mineral* 6:77-86, 1989
- 25 Riggs BL: Treatment of osteoporosis with sodium fluoride: An appraisal. In *Bone and Mineral Research, Annual 2* (Peck WA, ed.). Elsevier, New York, 1983, pp 366-393
- Delmas PD: Treatment of vertebral osteoporosis with disodium monfluorophosphate: Comparison with sodium fluoride. *J Bone Mineral Res* 5(suppl 1):S143-S147, 1990
- 30 Riggs BL et al: Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. *N Engl J Med* 322:802-809, 1990
- Mamelle N et al: Risk-benefit ratio of sodium fluoride treatment in primary vertebral osteoporosis. *Lancet* ii:361-365, 1988
- 35 Tietz NW, ed. *Textbook of Clinical Chemistry*. WB Saunders, Philadelphia, 1986, pp 1322-1323
- 40 Russell RGG: Bone cell biology: The role of cytokines and other mediators. In *Osteoporosis 1990* (Smith R, ed.). Royal College of Physicians of London, London, 1990, pp 9-33
- Tilyard MW et al: Treatment of postmenopausal osteoporosis with calcitriol or calcium. *N Engl J Med* 326:357-362, 1992
- 45 Meunier PJ et al: Can we stop bone loss and prevent hip fractures in the elderly? *Osteoporosis Int* 4(suppl 1):S71-S76, 1994

- Chapuy MC et al: Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. *Br Med J* 308:1081-1089, 1994
- 5 Neer R et al: Treatment of postmenopausal osteoporosis with daily parathyroid hormone plus calcitriol. In *Osteoporosis 1990* (Christiansen C, Overgaard K, eds.). Osteopress ApS, Copenhagen, 1990, pp 1314-1317
- Reid DM: Corticosteroid-induced osteoporosis. In *Osteoporosis 1990* (Smith R, ed.). Royal College of Physicians of London, London, 1990, pp 99-117
- 10 Lukert BP, Raisz LG: Glucocorticoid-induced osteoporosis: Pathogenesis and management. *Ann Intern Med* 112:352-364, 1990
- Reid IR et al: Two-year follow-up of bisphosphonate (APD) treatment in steroid-induced osteoporosis. *Lancet* ii: 1144, 1988
- 15 Ringe JD, Welzel D: Salmon calcitonin in the therapy of corticoid-induced osteoporosis. *Eur J Clin Pharmacol* 33:35-39, 1987
- 20 Rosen, CJ: Beyond one gene-one disease: Alternative strategies for deciphering genetic determinants of osteoporosis. *Calcif Tissue Int* 60(3):225-28, 1997.
- Keen et al.: Genetic factors in osteoporosis. What are the implicatins for prevention and treatment? *Drugs Aging (New Zealand)* 11(5):333-7, 1997.
- 25 Eisman: Vitamin D receptor gene variants: Implications for therapy. *Curr Opin Genet Dev* 6(3): 361-65, 1996.
- Ralston: The genetics of osteoporosis. *QJM – Monthly Journal of the Association of Physicians (United Kingdom)* 90/4, pp 247-51, 1997.
- 30 Cole et al.: Osteoporosis And the Interface Between Nutrition And Genetics. INABIS '98 – 5th Internet World Congress on Biomedical Sciences at McMaster University, Canada, Dec 7-16. <http://www.mcmaster.ca/inabis98/atkinson/cole0195/index.html>
- 35 Tokita et al.: Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *J Bone Miner Res* 11(7): 1003-09, 1996.
- Jouanny et al.: Environmental and genetic factors affecting bone mass. Similarity of bone density among members of healthy families. *Arthritis Rheum* 38(1):61-67, 1995.
- 40 Garnero et al.: Genetic influence on bone turnover in postmenopausal twins. *J Clin Endocrinol Metab* 81(1):140-46, 1996.
- 45 Johnston et al.: Pathogenesis of osteoporosis. *Bone* 17(2 Suppl):19S-22S, 1995.
- Fujita: Vitamin D in the treatment of osteoporosis revisited. *Proc Soc Exp Biol Med* 212(2):110-5, 1996.

- Gong et al.: Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12-13. *Am J Hum Genet* 59(1):146-51, 1996.
- 5 Houston et al.: Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. *Bone* 18(3):249-52, 1996.
- Cummings: Treatable and untreatable risk factors for hip fracture. *Bone* 18(3 Suppl):165S-167S, 1996.
- 10 Wasnich: Vertebral fracture epidemiology. *Bone* 18(3 Suppl):179S-183S, 1996.
- Riggs et al.: The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. *J Bone Miner Res* 10(6):991-6, 1995.
- 15 Wark: Osteoporotic fractures: background and prevention strategies. *Maturitas (Ireland)* 23(2):193-207, 1996.
- 20 Fleet et al.: The BsmI vitamin D receptor restriction fragment length polymorphism (BB) predicts low bone density in premenopausal black and white women. *J Bone Miner Res* 10(6):985-90, 1995.
- 25 Krall et al.: Vitamin D receptor alleles and rates of bone loss: influences of years since menopause and calcium intake. *J Bone Miner Res* 10(6):978-84, 1995.
- Livshits et al.: Population biology of human aging: segregation analysis of bone age characteristics. *Hum biol* 68(4):540-54, 1996.
- 30 Dalglish: The human type I collagen mutation database. *Nucleic Acids Res* 25(1):181-7, 1997 (citing <http://www.le.ac.uk/depts/ge/collagen/collagen.html>)
- Lonzer et al.: Effects of heredity, age, weight, puberty, activity and calcium intake on bone mineral density in children. *Clin Pediatr* 35(4):185-9, 1996.
- 35 Pereira et al.: Bone fragility in transgenic mice expressing a mutated gene for type I procollagen (COL1A1) parallels the age-dependent phenotype of human osteogenesis imperfecta. *J Bone Miner Res* 10(12):1837-43, 1995.
- 40 Sano et al.: Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. *Biochem Biophys Res Commun* 217(1):378-83, 1995.
- Burke and Olson, "Preparation of Clone Libraries in Yeast Artificial -Chromosome Vectors" in *Methods in Enzymology*, Vol. 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., Chap. 17, pp. 251-270 (1991).
- 45 Capecchi, "Altering the genome by homologous recombination" *Science* 244:1288-1292 (1989).

- Davies et al., "Targeted alterations in yeast artificial chromosomes for inter-species gene transfer", *Nucleic Acids Research*, Vol. 20, No. 11, pp. 2693-2698 (1992).
- 5 Dickinson et al., "High frequency gene targeting using insertional vectors", *Human Molecular Genetics*, Vol. 2, No. 8, pp. 1299-1302 (1993).
- Duff and Lincoln, "Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human APP gene and expression in ES cells", *Research*
10 *Advances in Alzheimer's Disease and Related Disorders*, 1995.
- Huxley et al., "The human HPRT gene on a yeast artificial chromosome is functional when transferred to mouse cells by cell fusion", *Genomics*, 9:742-750 (1991).
- 15 Jakobovits et al., "Germ-line transmission and expression of a human-derived yeast artificial chromosome", *Nature*, Vol. 362, pp. 255-261 (1993).
- Lamb et al., "Introduction and expression of the 400 kilobase precursor amyloid protein gene in transgenic mice", *Nature Genetics*, Vol. 5, pp. 22-29 (1993).
- 20 Pearson and Choi, Expression of the human α -amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. *Proc. Natl. Acad. Sci. USA*, 1993. 90:1057882.
- 25 Rothstein, "Targeting, disruption, replacement, and allele re-scue: integrative DNA transformation in yeast" in *Methods in Enzymology*, Vol. 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., Chap. 19, pp. 281-301 (1991).
- 30 Schedl et al., "A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice", *Nature*, Vol. 362, pp. 258-261 (1993).
- 35 Strauss et al., "Germ line transmission of a yeast artificial chromosome spanning the murine $\alpha 1(I)$ collagen locus", *Science*, Vol. 259, pp. 1904-1907 (1993).
- Gilboa, E, Eglitis, MA, Kantoff, PW, Anderson, WF: Transfer and expression of cloned genes using retroviral vectors. *BioTechniques* 4(6):S04-S12, 1986.
- 40 Cregg JM, Vedvick TS, Raschke WC: Recent Advances in the Expression of Foreign Genes in *Pichia pastoris*, *Bio/Technology* 11:905-910, 1993
- Culver, 1998. Site-Directed recombination for repair of mutations in the human ADA gene. (Abstract) *Antisense DNA & RNA based therapeutics*, February, 1998,
45 Coronado, CA.
- Huston et al, 1991 "Protein engineering of single-chain Fv analogs and fusion proteins" in *Methods in Enzymology* (ii Langone, ed.; Academic Press, New York, NY) 203:46-88.

- Johnson and Bird, 1991 "Construction of single-chain Fv derivatives of monoclonal antibodies and their production in *Escherichia coli* in *Methods in Enzymology* (JJ Langone, ed.; Academic Press, New York, NY) 203:88-99.
- 5 Mernaugh and Mernaugh, 1995 "An overview of phage-displayed recombinant antibodies" in *Molecular Methods In Plant Pathology* (RP Singh and US Singh, eds. ; CRC Press Inc., Boca Raton, FL) pp. 359-365.
- 10 Aiello et al., "Identification of multiple genes in bovine retinal pericytes altered by exposure to elevated levels of glucose by using mRNA differential display" *Proc. Natl. Acad. Sci. USA* Vol. 91, pp. 6231-6235 (1994).
- 15 Bauer et al., "Identification of differentially expressed mRNA species by an improved display technique (DDRT-PCR)" *Nucleic Acids Research* Vol. 21, No. 18 (1993).
- Bhanicha and Ven Murthy, "Characterization of Polysomes and Polysomal mRNAs by Sucrose Density Gradient Centrifugation Followed by Immobilization in Polyacrylamide Gel Matrix" *Methods in Enzymology* Vol. 216, pp. 168-179 (1992).
- 20 Braun et al., "Identification of Target Genes for the Ewing's Sarcoma EWS/FLI Fusion Protein by Representational Difference Analysis" *Molecular and Cellular Biology* Vol. 15, No. 8, pp. 4623-4630 (1995).
- 25 Cold Springs Harbor Laboratory Press, Cold Spring Harbor, New York, 1989
- Davis et al., "Expression of a single transfected cDNA converts fibroblasts to myoblasts." *Cell* 51:987-1000, 1987.
- 30 Diatchenko et al., "Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries" *Proc. Natl. Acad. Sci.*, Vol. 93, pp. 6025-6030 (1996).
- Ehrenfeld, "Initiation of Translation by Picornavirus RNAs", *Translational Control* Cold Spring Harbor Laboratory Press, pp. 549-573, 1996.
- 35 Hadman et al., "Modification to the differential display technique reduce background and increase sensitivity" *Analytical Biochemistry* 226:383-386 (1995).
- 40 Hanauske-Abel et al., "Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the GI-S boundary. Proposal of a role for EIF-5A in onset of DNA replication." *FEBS Letters* 386 pp. 92-98 (1995).
- Hirama et al., "Direct Purification of Polyadenylated RNAs from Isolated Polysome Fractions" *Analytical Biochemistry* 155, pp. 385-390 (1986).
- 45 Hubank and Schatz, "Identifying differences in mRNA expression by representational difference analysis of cDNA" *Nucleic Acids Research*, Vol. 22, No. 25, p. 5640-5648 (1994). Jefferies et al., "Elongation Factor-1a mRNA Is Selectively Translated

- following Mitogenic StimulatioW' The Journal of Biological Chemistry Vol. 269, No. 6, pp. 4367-4372 (1994).
- 5 Liang and Pardee, "Differential Display of Eukaryotic Messenger RNA by Means of the Polymerase Chain Reaction" ~S~cience, Vol. 257, pp. 967-971 (1992).
- Liang et al., "Distribution and cloning of Eukaryotic mRNAs by means of differential display: refinements and optimization" Nucleic Acids Research Vol. 21, No. 14, pp. 3269-3275 (1993).
- 10 Liang and Pardee, "Recent advances in differential display" Current Opinion in Immunology, 7:274-280 (1995).
- Linskens et al., Cataloging altered gene expression in young and senescent cells using enhanced differential display" Nuc. Ac. Res. 23: 3244-3251 (1995).
- 15 Lisitsyn and Wigler, "Cloning the Differences Between Two Complex Genomes" Science Vol. 259, pp. 946-951 (1993).
- 20 Mach et al., "Isolation of a cDNA Clone Encoding S-Adenosylmethionine Decarboxylase" The Annual of Biological Chemistry Vol. 261, No. 25, pp. 11697-11703 (1986).
- 25 Macejak et al., "Internal inition of translation mediated by the 5' leader of a cellular mRNA" Nature, Vol. 353, pp. 990-94 (1991).
- Mechler, "Isolation of messenger RNA from Membrane-Bound Polysomes" Methods in Enzymology Vol. 152, pp. 241-253 (1987).
- 30 Menaker et al., "A Method for the Isolation of Rat Submandibular Salivary Gland Polysomes on Linear Sucrose Density Gradients" Analytical Biochemistry 57, pp. 325-335 (1974).
- 35 Meyuhas et al., "Translational Control of Ribosomal Protein mRNAs in Eukaryotes" Translational Control,, pp. 363-388 (1996).
- Mountford et al., "Internal ribosome entry sites and dicistronic RNAs in mammalian transgenesis" TIG, Vol. 11. No. 5, pp. 179-184 (1995).
- 40 Ogishima, et al., "Fractionation of Mammalian Tissue mRNAs by High-Performance Gel Filtration Chromatography" Analytical Biochemistry 138, pp. 309-313 (1984).
Oh et al., "Gene regulation: translational initiation by internal ribosome binding" Current Opinion in Genetics and development pp. 295-300 (1993).
- 45 Pelletier et al., "Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA" Nature Vol. 334, pp. 320-325 (1988).
- Schena et al., "Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray" Science Vol. 270, pp. 467-470 1995).

- Shen et al., "Identification of the Human Prostatic Carcinoma Oncogene PTI- I by Rapid Expression Cloning and Differential RNA Display" Proc. Natl. Acad. Sci. USA Vol. 92, pp. 6778-6782 (1995).
- 5 Vagner et al. "Alternative Translation of Human Fibroblast Growth Factor 2 mRNA Occurs by Internal Entry of Ribosomes" Molecular and Cellular Biology, Vol. 15, No. 1, pp. 35-44 (1995).
- 10 Welsh et al., "Arbitrary primed PCR fingerprinting of RNA", Nuc. Ac. Res. 20:49654970 (1992).
- Zhao et al., "New primer strategy improves precision of differential display" Biotechniques 18: 842-850 (1995).
- 15 Sperling et al., "Abundant Nuclear Ribonucleoprotein Form of CAD RNA," Mol Cell Biol. 1985 Mar;5(3):569-75.
- Dimitrijevic & Wgner "A search for novel, naturally occurring antisense RNA systems"
- 20 Murphy, P.R. and Knee R.S. (1994) Identification and characterization of an antisense RNA transcript (gfg) prom the human basic fibroblast growth factor gene. Mol. Endo Vol. 8 no. 7 852-859.
- 25 Adelman, J.P. Bond, C.T., Douglass, J. and Herbert, E. (1987) Two mammalian genes transcribed from opposite strands of the same DNA locus. Science 235:1514:1313.
- Kimelman, Du Kirschner, M.W. (1989) An antisense m-RNA directs the covalent modification of the transcript encoding pibroblast growth factor in Xenopus oocytes
- 30 Cell, 59:687-696.
- Lazer, M.A. Hodin, R.A., Darling D.S., Chin, ww. (1989) A novel member of the thyoid/steroid hormone receptor family is encoded by the opposite strand of the rat c-erbA a transcriptional unit. Mol. Cell. Biol. 9:1128-1136.
- 35 Miyajima, N., Horiuchi, R., Shibuya, YI, Fuhushige, S., Mutsubara, K., Toyoshimak, Yamamoto, T (1989) Tow erba homologs encoding proteins with different T3 binding capacities are transcribed from opposite DNA strands of the same genetic locus. Cell. 57:31-39.
- 40 Kindy, M., McCormack, J. Buckler, A., Levine, R., Sanenshein G. (1987) Independent regulation of the transcription of the two strands of the c-myc gene. Mol. Cell. Biol. 7:2857-2562.
- 45 Celano, P., Berchtold, C., Kizer, D., Weararatna, F., Nelkin, B., Baylin, S., Casero, R. (1992) Characterization of an edogenous RNA transcript with homology to the antisense strand of the human c-mye gene. J. Biol.. Chem. 267:15092-15096.

- Armstrong, B.C., Krystal, G.W. (1992) Isolation and characterization of complementary DNA per N-cym, a gene encoded by the DNA strand opposite to N-myc Cell Growth Differ. 3:385-390.
- 5 Taylor, E.R., Seleiro, E.A.P. Brickell, P.M. (1991) Identification of antisense transcripts of the chicken insulin-like growth factor II gene. J. Mol. Endocrial 7:145
- 10 Mitchell, P.J., Carothers, M., Han. J.H., Harding, J.D., Kas, E. Vendia, L., Chasin, L.A. (1986) Multiple transcription start sites, Dnase I-hypersentive sites, and an opposite strand exon in the 5' region of the CHO dnfr gene. Mol. Cell. Biol. 6:425-

- A search for novel, naturally occurring antisense RNA systems, N. Dimitrijevic,
5 E.G.H. Wagner, Dept. of Microbiology, SLU (Swedish University of Agricultural
Sciences), Genetic Center, P.O. Box 7025, S-75007 Uppsala, Sweden

Antisense RNA control of gene expression has been demonstrated in many
bacteria, whereas only few cases are known in eukaryotes. All antisense RNAs
10 identified to date have been found fortuitously. Therefore, our goal is to develop a
novel strategy for targeted identification of naturally occurring antisense systems. The
approach is based on the complementarity between antisense and target RNA over a
significantly long stretch of nucleotides. The method used is briefly described here.
Total cellular RNA is extracted. Part of the RNA pool is immobilized on a
15 membrane, another part converted into cDNA after ligation of oligodeoxynucleotides
to the 3' ends. The use of biotinylated, complementary oligos for cDNA synthesis
allows immobilization of a "minus" strand to streptavidin-coated magnetic beads. A
second set of oligos is ligated to the cDNA at the previous 5' end of the RNA. Plus
strands are eluted from the bound strands and hybridized to the membrane-bound
20 RNA. Since the cDNA strand used has the same polarity of the RNAs, only cDNA
sequences that can bind to complementary RNAs should be retained. PCR
amplification and subsequent cloning of PCR-fragments is followed by sequence
analysis. To test whether cloned sequences are correctly identified, probes are
generated in sense and antisense direction. Positive clones will be structurally and
25 functionally characterized. In order to work out this method, we started using a
bacterial strain (*Escherichia coli*), containing plasmid R1 that regulates its copy
number by antisense RNA. Previous work has identified both antisense (CopA) and
target RNA (CopT) of R1 intracellularly. This procedure, if feasible, will then be
used to screen for antisense RNA systems in other organisms.

WHAT IS CLAIMED IS

1. A method or process for identifying genes whose expression is responsive to a specific cue or cues including the steps of:
 - (a) applying a cue to an organism or tissue or cells;
 - 5 (b) isolating specific cellular fractions from the tissues or cells subjected to the cue;
 - (c) extracting the mRNA from the cellular fractions; and
 - (d) differentially analyzing the mRNA samples in comparison with control samples not subjected to the cue to identify genes that have responded to the cue;
- 10 wherein the tissue or cells comprises bone cells that retain being bone cells in a culture.
2. A method as set forth in claim 1, wherein genes are identified at the translation level; genes regulated at the transcription level; genes regulated by RNA stability; genes regulated by mRNA transport rate between the nucleus and
- 15 cytoplasm; genes regulated by differential splicing; and genes regulated by antisense RNA.
3. The method of claim 1 wherein the cue comprises a stress inducing element which comprises mechanical stress.
4. The method of claim 1 wherein the bone cells comprise calvaria cells.
- 20 5. A method for determining risk of developing a physiological or disease state based upon presence or increase from normal cells or absence or decrease from normal cells of mRNA or protein from a gene shown to be up regulated or down regulated in a mammal by a method of claim 1 comprising:
 - (a) determining the level or status of mRNA in cells of said mammal; and/or
 - 25 (b) determining the level or status of corresponding protein in cells of said mammal; and
 - (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a
 - 30 transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a

presence or increase from normal cells, or an absence or decrease from normal cells, of mRNA or protein and thus risk of developing a physiological or disease state.

6. A method for testing a medicament for or a gene therapy approach to a physiological or disease state or other factors causing or contributing thereto or to symptoms thereof based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein of identified genes comprising a method as claimed in claim 5 additionally comprising: (a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or increase from normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

7. A method for treating, preventing or controlling a physiological or disease state comprising a method as claimed in claim 5 and additionally comprising administering a medicament or treatment therefor or for a cause thereof or a symptom thereof.

8. The method of claim 6 wherein the medicament or treatment comprises the protein, a functional portion thereof, a vector expressing the protein or a functional portion thereof, or an inhibitor of the protein or of a functional portion thereof, or an inhibitor of a nucleic acid encoding the protein or a functional portion thereof.

9. The method of claim 5 further comprising:

- (d) determining the level or status of a second gene mRNA in cells of said mammal; and/or
- (e) determining the level or status of protein expressed by a second gene product in cells of said mammal; and
- (f) comparing said level or status of that mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining risk.

10. The method of claim 9 wherein steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vivo* and/or steps (a) and/or (b) and optionally (d) and/or (e) are carried out *in vitro*.

11. The method of claim 9 wherein the determination in step (a) and
5 optionally in step (d) is effected by employing
(i) a nucleic acid sequence corresponding to at least a part of the gene encoding at least part of the protein and optionally a second nucleic acid sequence corresponding to at least a part of the second gene encoding at least part of the second protein;
(ii) a nucleic acid sequence complementary to the nucleic acid sequence(s) of (i);
10 or
(iii) a primer or a primer pair hybridizing to the nucleic acid sequence(s) of (i) or (ii).

12. The method of claim 9 wherein the determination in step (b) and optionally of step (e) is effected by employing an antibody or a fragment thereof that
15 specifically binds to the protein and optionally by employing a second antibody or a fragment thereof which specifically binds to the second protein.

13. A gene identification method comprising: preparation of probes from a model system; analysis of DNA chip hybridization; sequencing of clones showing differential expression; and optionally full-length cloning of clones of interest;
20 wherein the model system comprises bone cells which retain their characteristic thereof in cultures which have mechanical stress or a lack thereof applied thereto.

14. The method of claim 13 wherein the bone cells comprise a calvaria primary culture.

15. A method for determining risk of developing osteoporosis or low or
25 high bone density or other factors causing or contributing to osteoporosis or other conditions involving mechanical stress or a lack thereof, based upon presence or increase or absence or decrease from normal cells of mRNA or protein from a gene shown to be up regulated or down regulated by a method of claim 13 in a mammal comprising:
30 (a) determining the level or status of mRNA in bone cells of said mammal; and/or
(b) determining the level or status of corresponding protein in bone cells of said mammal; and

- (c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a presence or increase from normal cells, or an absence or decrease from normal cells, of mRNA or protein and thus risk.
- 10 16. A method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or lower levels of osteoblasts and chondrocytes or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells of mRNA or protein from 608 comprising:
- 15 (a) determining the level or status of mRNA in bone cells of said mammal; and/or
(b) determining the level or status of corresponding protein in bone cells of said mammal; and
(c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or
20 protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining a
25 presence or increase from normal cells of mRNA or protein and thus risk.
17. A method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of imbalance as to osteogenic and chondrogenic cells or other conditions involving mechanical stress or a lack thereof, based upon absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein from 405 in a mammal
30 comprising:
- (a) determining the level or status of mRNA in bone cellsof said mammal; and/or

(b) determining the level or status of corresponding protein in bone cells of said mammal; and

(c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

18. A method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or of being susceptible to environmental factors or other than genetic factors of osteoporosis or of predisposition of bones towards susceptibility to environmental factors, or less lymphoid cells, or osteoporosis, or other conditions involving mechanical stress or a lack thereof, based upon presence or increase from normal cells or absence or decrease from normal cells of mRNA or protein from 274 in a mammal comprising:

(a) determining the level or status of mRNA in bone cells of said mammal; and/or

(b) determining the level or status of corresponding protein in bone cells of said mammal; and

(c) comparing said level or status of mRNA and/or protein with the corresponding level in normal cells; wherein the term "level" denotes the amount of mRNA or protein produced; and, the term "status" includes that the gene, mRNA, protein or a transcription control element, including a promoter/enhancer sequence, may bear a mutation, deletion or any other modifications which would affect the overall activity of the gene when compared to the wild-type normal gene product, including post-translational modifications of the protein, and from the comparing determining an absence or decrease from normal cells of mRNA or protein and thus risk.

19. A method for testing a medicament for or gene therapy approach to osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, based on absence or decrease from normal cells or presence or increase from normal cells of mRNA or protein comprising a method according to any one of claims 15-18

and additionally comprising: (a') administering the medicament or the gene therapy; and from the comparing determining an absence or decrease from normal cells or presence or increase from normal cells of the relevant mRNA or protein and thus efficacy of the medicament or the gene therapy.

5 20. A method for treating, preventing or controlling osteoporosis or other conditions involving mechanical stress or a lack thereof, comprising a method according to any one of claims 15-18 and further comprising administering a medicament or treatment for osteoporosis or a cause thereof or a symptom thereof.

 21. A composition comprising a gene or portion thereof or a protein or
10 portion thereof expressed by the gene or portion thereof or an antibody or portion thereof which binds to the protein or portion thereof, wherein the gene is identified by a method as claimed in claim 1 or 13.

 22. An osteoporosis or mechanical stress or lack thereof model comprising bone cells which retain their characteristic thereof in culture with mechanical stress
15 applied thereto or an absence of mechanical stress applied thereto.

 23. An isolated nucleic acid molecule: encoding the herein identified protein 608 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto; or encoding the herein identified protein 405 or a functional portion thereof or a polypeptide which is at least
20 substantially homologous or identical thereto; or encoding the herein identified protein 274 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.

 24. The isolated nucleic acid molecule of claim 23: encoding human protein 608 or a functional portion thereof; or encoding human protein 405 or a
25 functional portion thereof; or encoding human protein 274 or a functional portion thereof.

 25. The isolated nucleic acid molecule of claim 23: comprising SEQ ID NO: or a functional portion thereof; or comprising SEQ ID NO: or a functional portion thereof; or comprising SEQ ID NO: or a functional portion thereof; or
30 comprising a nucleotide sequence as shown in the Figures or a functional portion thereof.

 26. A vector comprising an isolated nucleic acid molecule of any one of claims 23-25.

27. A composition comprising the vector of claim 26.
28. A probe or primer which specifically hybridizes to an isolated nucleic acid molecule of any one of claims 23-25.
29. An expression product of the isolated nucleic acid molecule of any one
5 of claims 23-25.
30. An isolated polypeptide: herein identified as protein 608 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto; or herein identified as protein 405 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto; or
10 herein identified as protein 274 or a functional portion thereof or a polypeptide which is at least substantially homologous or identical thereto.
31. The isolated polypeptide of claim 30: which is human protein 608 or a functional portion thereof; or which is human protein 405 or a functional portion thereof; or which is human protein 274 or a functional portion thereof.
- 15 32. The isolated polypeptide of claim 28: comprising SEQ ID NO: or a functional portion thereof; or comprising SEQ ID NO: or a functional portion thereof; or comprising SEQ ID NO: or a functional portion thereof; or comprising a polypeptide sequence as shown in the Figures or a functional portion thereof.
33. A composition comprising the isolated polypeptide of any one of
20 claims 30-32.
34. An antibody elicited by a polypeptide of any one of claims 30-32 or a functional portion thereof.
35. A composition comprising the antibody or functional portion thereof of claim 34.
- 25 36. A method for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering a polypeptide as claimed in any one of claims 30-32.
37. A method for preventing, treating or controlling osteoporosis or bone
30 density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering a vector as claimed in claim 26.

38. A method for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering a composition as claimed in claim 21.

5 39. A method for preventing, treating or controlling osteoporosis or bone density or other factors causing or contributing to osteoporosis or symptoms thereof or other conditions involving mechanical stress or a lack thereof, comprising administering a gene or functional portion thereof or a polypeptide comprising an expression product of the gene or functional portion of the polypeptide or an antibody
10 to the polypeptide or a functional portion of the antibody, wherein the gene is identified in the model of claim 22.

40. The method of claim 39 wherein the gene identified in the model is CMF2-45 or CMF2-224.

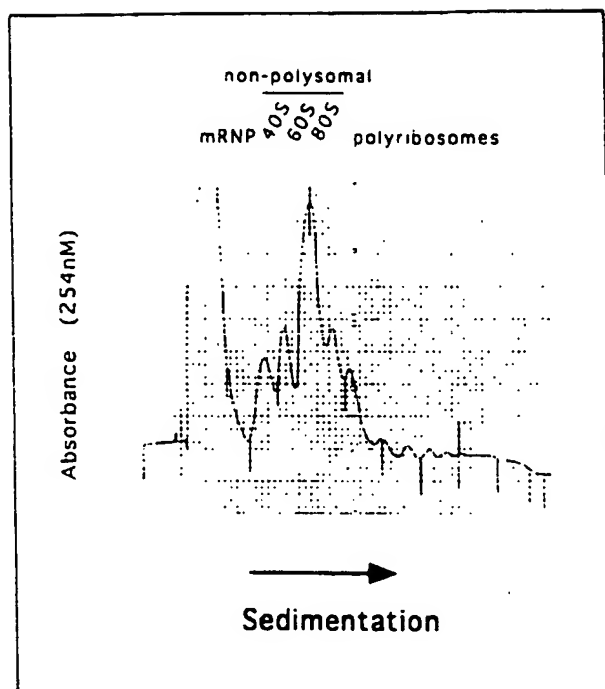
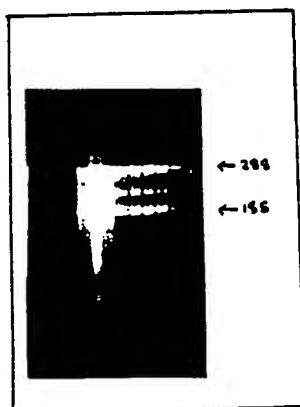
41. A method for preparing a polypeptide comprising expressing the
15 polypeptide from the vector of claim 26.

42. A method for preparing a polypeptide comprising expressing the polypeptide from a gene identified in a method as claimed in any one of claims 1 or 13.

43. A method for advancing research in or studies of bone development
20 comprising a method as claimed in any one of claims 1 or 11.

44. A method for affecting a gene identified by any one of the methods of claim 1 or 11 comprising contacting cells containing the gene with a compound to which the gene responds.

45. The method of claim 44 wherein the compound comprises estrogen or
25 a derivative or precursor thereof.

*FIG. 1A**FIG. 1B*

2/183

FIG. 2

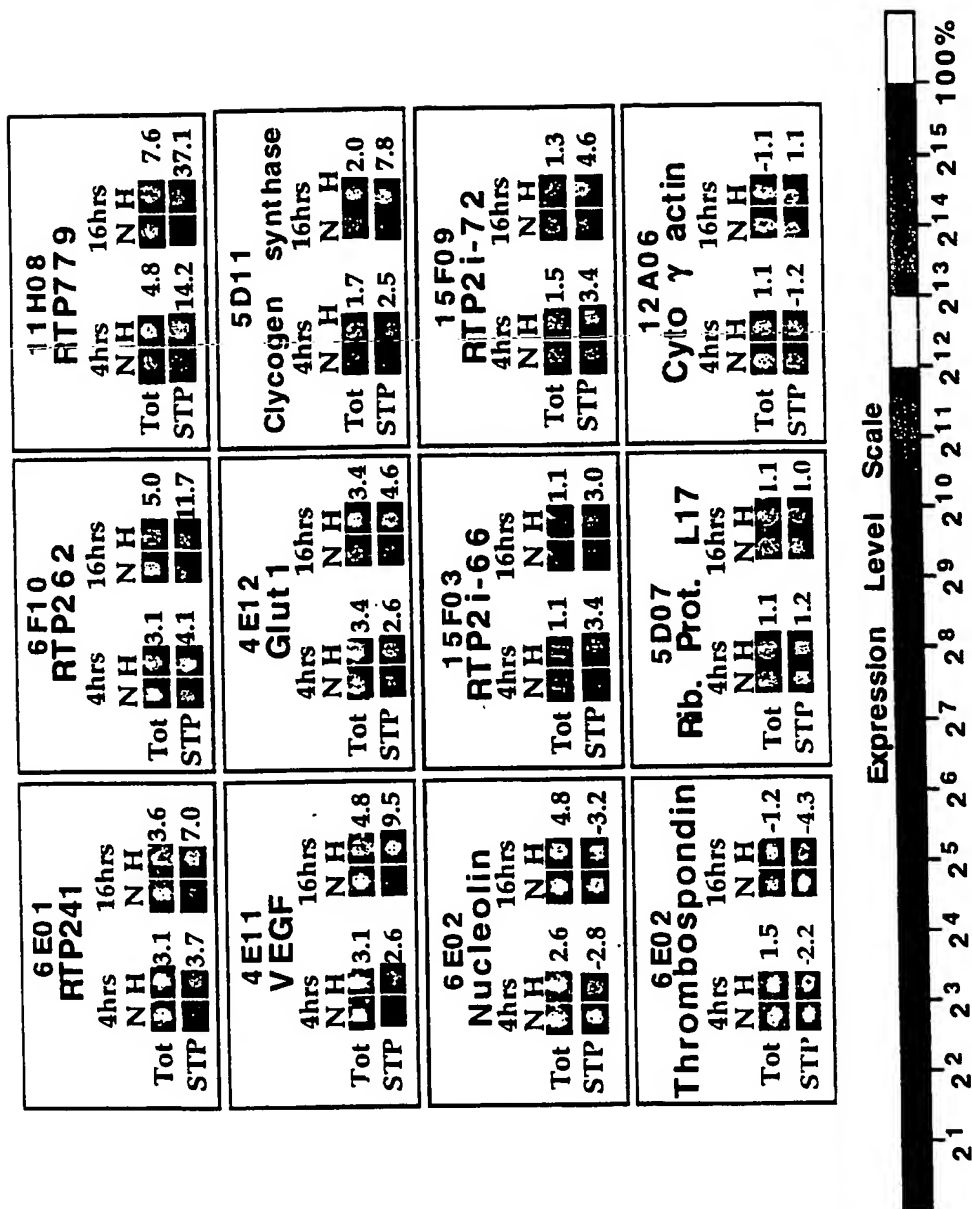


FIG.2A

GENE NAME	-Ca ²⁺ MF	+Ca ²⁺ MF	INDO. MF	(103) +PGE ₂	(104) +PGE ₂	Q.B.I. SEQUENCE NO#
(+) UPREGULATED, (+) DOWNREGULATED	109	110	111	107	116	
	POLYA+	POLYA+	POLYA+	POLYA+	POLYA+	
1 α 2U GLOBULIN-RELATED PROTEIN	-3.8	-4.9				10C12
2 α -ACTIN, CARDIAC	-3.6	-3.7				9C6
3 γ -ACTIN, CYTOPLASMIC	-3.5	-1.7		-1.1	-1.2	1D6
4 ALKALINE PHOSPHODIESTERASE RB13-6 Ag	-3.9	-3.3				11C8
5 AQUAPORIN (AQP1), CHANNEL INTEGRAL MEMBRANE PROTEIN	-3.5	-2		-1.1	-1.1	6D3
6 α 2 RECEPTOR/ LDL RECEPTOR/ LPR / A-2 MACROGLOBULIN RECP	-3.6					7A6
7 COLLAGEN TYPE XII A-1	-3.2					3D2
8 COMPLEMENT COMPONENT C3	-3.9	-4.7		-2.3		4B3
9 PREPRO COMPLEMENT COMPONENT C3	-3.3					4B8
10 RAT pRC201 m RNA PREPRO COMPLEMENT C3 (X52477)	-2	-3.8				4B5
11 SYNTROPHIN 1 MUS MUSCULUS (U00677)	-2					13C4
12 CYTOCHROME OXIDASE C	-2.5	2.3				2F6
13 DEST (2-144)	-3.6	-2.5				2D12
14 DEST (274)	-2.1					3G10
15 DEST (608) HOMOLOG NCAM-1	-4					7C8
16 DEST (AA240223)	-2.2	-2.1				2G8
17 DEST 700	-2.6					8C4
18 DEST KIA0183	-2.2					5B9
19 DEST (2-197) SIMILARITY TO eig3 (Y10624)	-3.8	-2.3				11A5
20 ENDOTHELIN CONVERTING ENZYME	-3.6	-2.5				14C8
21 eig25 METHYL STEROL (U60205)	-2.7	-2.4		-2.3	-2.1	5B8
22 FILAMIN (OR REGION BETWEEN FILAMIN & GPI)	-3.5					4G9
23 H.SAPIENS m RNA FOR GIGF (X78974)	-3			-2.2	-2.2	3E8
24 HIGHLY CHARGED AMINO ACID SEQ (X59131)	-3.7	-5.1				9C4
25 KIDNEY AMINOPEPTIDASE MKIDNEY Zn-PEPTIDASE	-2.3	-5.8				11A12
26 MOUSE THROMBOSPONDIN 1 (M87276)	-2.6	2.6				7C9
27 MYOSIN HEAVY CHAIN A	-3.2					5A1
28 OSTEOECTINE	-3.4	-2.6				2H12
29 NOVEL PROTEIN AHNAK NUCLEOPROTEIN (M80899)	-2.8					14D6
30 RAT mRNA FOR FIBRONECTIN +[67	-3.3	-4.1				13D8
31 SARP 1 (AF-017989)	-3.7	-2.1				(2, 45)
32 SECRETORY PROTEIN CONTAINING THROMBOSPONDIN MOTIFHS	-3.3					2H11
33 STEAROYL CoA DESATURASE (PROTEIN KINASE)	-3.6					6G10
34 TENASCIN	-4					2H12

FIG. 2A-1

1. >cal2-294 = a 2u globulin-related protein
TACTGTTTGTTCAGAGGTCTTCTGGAAAAATACCATGGCAAACTGGTCGTAGTCAGTG
TCGGCCACTTGACATCGTAGCTCTGTATCTGAGGTAGCTGTGAATATCCCCAGGGTG
AACTGGCCAGGCTGGAGCTTGGAACGAATGTTCTGATCCAGTAGCAGACCCCTGGCCC
CTGACGAGGATGGAAGTGACGTTGTAGCTATTGTCTTCTGTAGCTCATAGATGGTGCTG
T
2. >cal1-42 = g-actin, cytoplasmic
ACATGGCTGGGTATTGAAGGTTTCAAAACATTATCTGCGTCATCTTCTCT
CTGTTAGCTTTGGGTTTCAGGGGGCCCTCGGTCAGAAAGCACCCGGTGCTC
CTCAGGGGCCACACGCAGCTCGTTGTAGAAGGTGTGGTGCCAGATCTTCT
CCATGTCGTCCTCCAGTTGGTGACAAATGCCGTGCTCAATAGGGT
3. >cal2_54 = a-actin, cardiac
ACAGGTCTTTGCGGATATCGATGTCAACACTTCATGATGCTGTTATAAGTAGTTTCGTGAA
TGCCGGCAGACTCCATACCAATGAAAGAGGGCTGGAAGAGAGTCTCAGGACAGCGGAAC
GCTCGTTGCCAATGGTGATGACCTGGCCATCGGGAAGTTCGTAGCTCTTCTCCAGAGAGG
AGGAAGATGCAGCGGTGGCCATCTCATTTCTCAAAATCCAGGGCGACATAACACAGTTTTT
CTTTGATGCTCTCGGACAAATTTACGTTTCAGCGGTGGTGACAAAGGAGT

FIG. 2A-2

4. >cal2_224 = Alkaline phosphodiesterase
 ACCNAGGACCTGCCCCCTCGAATCAGGACTCGTAATACCTCAGGACTTCTTTACCTTTA
 ATCCGAAGAGATTGTCAGAGACCTCAGCTGTCGAAATCCGATCAACACTTCAAAACCCT
 ACTTGACTCCGGATTGCGGAAACGACTGCNCTATGCCAAGAACGTCAGGATTGACAAAG
 TTCATCTAATGGTGATCGGCTATGGCTGGCTTACAGGAACCTAAGGGAGTCCAACTGCCG
 AATGCGGAANNACNGTTACAACATATGAATTTAAANNTTGGANGCAATCTTTTGGCAC
 ATGGACCCNGCTTTAANGAGAANACTGTTNTCCAACCATTTGAAACACATCGAAGTCTATA
 NTCTACTGTGTGATCTTCTGCACNTCCN
5. >call-582 = AM2 receptor/LDL receptor related ; LRP/a-2-
 macroglobulin receptor
 ACCCTTCTGCCCTGTCCCTGTTGAAGACCTTGATGTCTTTAAGCTGCACGCCNATGCCCGT
 TCTCAGAGGCACGGAGTCTGTGGCATTTGCCATCCACGCTTGATGGAGCCGTTGGC
 GTGAGTTCTGTCACTCCAGTAGATGAAATCCTCAACACACAGACACAGAGAACATGTCCAT
 GTTGTTACTGGACAGGACCACCTCACGGTTCTCACCCGTTCTCCAGATCGATACGTTCCGAT
 CTTGTCCATCCGGGCATCACACCATAGAGCTTGCCGCCCTGATAGTCTACTGAGATGCC
 ATTGGGCCAGCTGATGCTGACATTAACAGCACCACTCTCTGTGTGCCGTCAAGGCGGAGA
 CCGCTCAATCCGTGGGTAGTGACCCCACTCAGTCCAGAACAAAG

FIG. 2A-3

6. >cmf1-519 = Aquaporin (AQA1); Channel integral membrane protein
28;
ACCATGGCACCAACTGGCTGTTTCCCTCTAGTCTCCCTTGCAGTGCAGATGTGACGTGTG
TGTTTATTAAAGAGCACTGGGCTATTGCAGCGTCAATGTCTGAGGAAAGAACGCTAGAC
ATGCAACAGACCCACAGACAGATGCCCATGCCGGGCACACAGGGGTTTGGATGCCCTAGT
TCATCATGATCAAGGAGGCCACTCTTGCTGCTTATGAGCTGCTGGCTGGATGAATTT
GACCAGAGCCTGGACAATCTGAAGGGGCTCACTATGTGACTCCAGGCACAGTCTCCTTAT
TGCAAGGACCTGATGCTGTGGCTTCTGCTATAGCCCAAGAACATCTCAGAGTGCATTGAG
CTCAGGGCTTGCAATTTAGCTCTCTGGGTTATTCTATTCAATCCACCAAGCCAGAGCAGC
TCTACCACTGTGCCGTTAACCATGTGCGTGAACCGAGACCCACATCTTCAGGTGCTTAGAA
GCAGCAGAATAATCAGGAGGCCATTGACCACTGGCATTAGT
7. >call-138 = Collagen type XII a-1
ACTTAAATGTGACAGATCTGAAAACCTTACCAGGTTGGATGGGACACCTTCTGTGTCAAAT
GGTCACCTCACCGGGCAGCGACCTCTTACAGGCTAAAGCTGAGTCTGCCGATGGAAACCA
GGGACAAAGAAATAACAGTGAGAGGATCAGAGACCACTGCTTCACTGGCTCTCTCCC
CGGAGGCTGAGTATGGCGTTACTGTTTGTGACAGACACCAATCTCGAGGGGCCGGGTG
TCCCATCAAAGAACAGACGACTGTGAAACCAACAGAGGCTCCACAGAAACCGCCACGC
CTTCACCTCCTCCCACTATCCACCTGCCCGTGATGTATGCAAGGGGCCAAGGCAGATA
TTGTGTTCTGACGGATGCCCTCTTGGAGTATTGGAGATGACAAATTTTAAACAAGTTGTAA
AATTATTTTAAATACTGTGGGGCCCTTGTATGAAATCAGCCCTGCTGGGATTCAGGTTT
CTTTGTGTCAGT

8. *FIG. 2A-4*

>cal2_155 = Complement component C3
 ACTTGGAAACTTGGATGCTACTATGTCCATCCTGGACATCTCCATGATGACTGGCTTTA
 TTCCAGACACAAACGACCTGGAACTGCTGAGCTCTGGAGTAGACAGATACATTTCCAAGT
 ATGAGATGGACAAAGCCTTCTCCAACAAGAACACCCCTCATCTACCTAGAAAAGATCT
 CACACTCCGAAGAAGACTGCCCTGTCCTTCAAAGTCCACCAAGTTCTTTAACGTGGACTTA
 TCCAGCCGGGTCGGTCAAGTCTACTCCTACTACNATCTAAAGGAGTCNTGCACCCGGT
 TCTATCATCCGGAGAAAGGACGATGGAATGCTGAGCAAGCTGTGCCACAATGAAAATGTGCC
 GCTGTGCCGAGGAGAACTGCTTCNTGTCATCAGTCACAGGATCAGGTCNGCCTGAATGAAC
 GACTACACAAGGCTTGTGAGCCTGGAGTGGACTACGTGTACCTCGGCCGCGACCACGCTG
 CTGAGCCTTGANCCGGGGAACAANAACNGCCCACTCTGGGAGGCCCTTTCNAAGT
 GGTGCTGGGACAAAGGATCCCCCTGCTCTNGTGTCTCCCTCCNGACTTATTGATTTCTTTC
 CNTTAACCTGTCTGGGACAGCCCTTTCGACCCCTNTGGCNAAGANAACACCCCCNCCCTTT
 AAAATCANCCCCCTCACTGGGTTTTCGACCCCTNTGGCNAAGANAACACCCCCNCCCTTT
 ATGCCCCCNGGTGTTGNCCTTNCCTCGTTATGGGCC

9.

>cal2_162 = Cytochrome oxidase subunit 1, mitochondria
 ACGATCCCTGTAGGCCCCCTACTGTGAATAAGAAGATAAACCCCTAAGGCTCATAATATG
 GCGGGGATCATTTGATATTCCCTCCATGTAGTGTAGCGAGTCAGCTGAATACTTTACG
 CCTGTAGGAATTGCGATAATTATAGTGGCAGATGTAAAGTAGGCTCGGGTGTCTACATCT
 AGGCCCTACTGTGAATATGTGATGTGCTCATACATAAACCCCTAGGAAGCCAATAGATATT
 ATGGCTCATACCATACCTATATATCCGAAGGTTCTTTTTCAGAGTAATAGGTAAC
 ACATGTGAAATAAATTCCAAACCCCTGGAAGAAATTAAGATGT

FIG. 2A-5

10. >cal1-274 = DEST
ACCGTGTGCCACTTTAATGGAAGACAGCTTGATATAACAAACGGCCT
TCAGGGTTATTACACACAGGAGGATCACTCTCTAGGTAGTCCATC
AAACTCCACTAAGCCAGACAAGGTATTGTAATGTTGGAGTTGGGATGAT
TGGTGAGAAATGTGGTTCTGAGTCCTCAGAACTCCACGGCCTTCTGTGAG
T

11. >cal2_144 = DEST
ACACACTGCCCACTCTCCTCTTGGCAGCAGCCCTGCCACTTTCTTACACTGCCAGCCAC
ATGTGGACATGGGCTACTCATGACTGTGGAATTCTCTTGGTGCCACCTTATTCCTCAGTG
GTGCTGGTATGCCCAATGGATAGCATCAAGATATGCCAATGTGTTTTTGTGGGCCAGTT
GGTTCCTTGTGGGATCTGAGAGTGGTTGACTCCAGTCCAGGGTCTTTCAGTTTCTATTTC
ACTCTGTCTGAGCAACACAGCTTCAATGCCCCGGATGAGTGGAACATATCCATGCTCAGTAA
TCTTCTGCGACATCAGGGTTTCTCTGTGTTTCATGAATGGAATTGGTTCCCTTCCCTCCA
CAACCACAAATTCAATCTTCAAAGAAGACTTGCTTTGGTCCCTCTGTCCAGTGGTCTTC
AAGGAGCCCTAGTCCCAGAGTCCACTCTCCAGGTGAGCCCACTCCCAGAGGGTCTTTC
CCACTGAATGGGTCTTCCAGCTGCTGGAAAGTGGCTGTGNGAACCCNGCCTCTTAAGACA
CAGTGGCTTCAGTTGCCAATGTTTCAGAGGGAAAGACCTCTTCTGGGGAGATNANTGGGAA
GGGAAGGGATAAAAATGACCTCCTGTTTTCTTGGTGTAAAGTTTCATTGAATTTTAAATG
GCGANGGTCTCACACTGGTTAACCACTTAAAGANACCAATCACTTTCNNGGTTGGCCATN
AAATGTTGAATGTCTTTGGGCTCAGTTTCATTCA

FIG. 2A-6

12.
>call_608 = DEST; homology to Neural cell adhesion molecule 1
ACCTCTGGTCCTATAGCCACTGTCACTGAGCTTGGGCTACATTGTTGAGCACTGTCCCAT
CAGGCAACTCCAGGATACCTCAGGCACAGGGAGCCAGAGGCCCTTGCACTCAACTTGGA
AATCTTTCCCATGGAGCACTTGCTTCTTAAATACTGCTTCTGTTCAATTTGGCAGGTG
TCAATCTCAGGCGACATGCATCAGGACTAGGTCACTCTCCCATTTTGTCTTGGCCACAC
ATAAGTAGTCACCGGTCCTTTTCCGTCACCTGACCCACCAAGGATCCATTTGGGT
AGACGTGGATTGCGCTGCCCATTTCTGTGCCACTGGTCGATGACAGCCTTGATGGCAGCC
TCCAGATTATTCTAGGCTTTGGATCCCCAGTAGCTGAGCAGTTCACTAGTAATTCTCAC
CCAAATTCACCTCAGTCCATGTCTGAGAGGCAGTTTCTATCCTGGGGTTGTCTCTCCCT
CTTCCACAGTAAGAACACTACCCCTTCTCTGAGCCTGAGGAGCTGGTGGCAATGCACCTC
ATAAGTCCCCCTCACTGAAGGACGATGCTTCTTATATACAGAGTTCATTTGGATACAAG
AAAAATCTGGGAATGAGTCAACTGCNATGGTTTGTAGTTCACTCCCATCAATAAGGACCC
AGTGAACACTAGGGTGGGGGAATTCCCTTTGTCAGTGCAGGGCATTTCAAACCTTCCACCTA
AAAACCCCAACGATNGGCTTNCCTCTTTTGGNTCCTATAATGAACAGGGGAACNGTTGAA
TTAACTTTTTTCTTTAACCCCAACANTTGAATCCTGGNCCANAATGGGTTGCCCTGGCC

FIG. 2A-7

13.
 >cal2_197 = DEST; some similarity to: erg3 (Ascobolus Immersus;
 Y10624)
 ACCTGTTCCCCACCAGCGCCGAAGACTGCAAAATTCACAGGCAATTTCTTCTACAACACTACA
 TGATGGGGAATTGAGTTCAACCCCGCATCGGAAAGTGGTTTGACTTCAAGCTGTTCTTCA
 ATGGCGCCCGGGGATTTGTGGCCTGGACTCTTATCAACCTGTCTTGTGCTGCCAAGCAGC
 AGGAGCTTTATGGCCATGTGACCAACTCCATGATTTTGGTCAATGTCTTACAGGCCATCT
 ATGTGTAGACTTCTTCTGGAACGAAACCTGGTATCA
14.
 >cal1-405 = DEST=KIAA0183
 ACATCAGTCCCTTCTTGTGCTGTGCTCAGCCACTCTCAGCACCTCAGGT
 GCAACGGGGGCAAGGAGGTGTGGTGATATCCATGTGGTTCCCTGGTGGG
 CATTGACAGAGGACCGGGATTGGCTGAACAGTGCCCATGTGGCTCCCTT
 TGCCTTCAGCTAGCTGGCTTCCGAAAGAGGAGTGATGAACCTTCTACC
 TTACTTGGTCTCCTGGGTCTCCCGTGCCAGTGCTCTGCATGGCTTCC
 TGTCTTCTCCAGCCAATCTTGTTCCTCCGTGATGTGGTTGGTTGCCCTC
 CACTGCCCTCATCGTTGTCTGATGAGGACGAGGTAGACgAgCCAGGGGA
 GTAgCGGGAATCCACGTGATTGGGGCTGCCGCTCTGGGCCAGGTTGATGG
 GCGAAGAAACGCTCATACAgCGGTGTGTGCTTCCCTCGTGAGGAAATGT
 TGCTGTAGCTGTTCTGCTCTGTCAAGTTCTTCCCGCTAGAgTCCAGGGCG
 AAGGCAGGCTCAgGGAAGCTGTATGGGTATGGAACCTGGCCTGGGGTGCC
 CCTGGGCTGGGCACCTGTTGGACCGGGCTTTGCTTGC

15. *FIG.2A-8*

>call14c8 = Endothelin converting enzyme
 ACGGGACAAAGAAAGACCTGTCTTCCCGCTGGAAAGTTTGTGGTGAGTGACACAGAAACA
 ACCTGGGCTTTGGCCTGGGCCCATGTTTGTGAAGCAACCTTTGCGGAGGACAGCAAGA
 ACATAGCCAGCGAGATCATCTGGAGATCAAGAAGGCATTCGAGGAGAGCCTGAGCACCC
 TGAAATGGATGGATGAAGATACTCGGAGGTCAGCCAGGAGAAAGCGGACGCCATCTACA
 ACATGATAGGCTACCCCAACTTCATCATGGACCCCAAGGAGCTGGACAAAGTGTTCATG
 ACTACACAGCAGTTCCTCGATCTCTACTTTGAGAACGCCATGCGATTTTCAACTTCTCAT
 GGAGGTCACAGCCGACCAAGCTCAGGAAAGCCCCAACAGAGATCAGTGGAGTATGACCC
 CGCCCATGGTGAACCTCTACTCGCCCAACCAAGAACGAGATTGTTTCCAGCTGGAA
 TCCTGCAGGCGCTATTTATNCCCGCTCTCGCCCAACGCCCTTGAACCTTGGTGGTATCC
 GGGTCTTTGTTGGCACNAACTGACTCCTGCTTTCNACGATCAGGGCNGGAGTTTGACAA
 GGATNGGAANCTCCCGCCTGGTTGGGAAAAACNCCTCGGTGGAGCTTCANCCNCANA
 ACCAATGCTTGGTTTCAACCTTTCAAACCTCCAACNCCANGGGGGGCCCGTTCCC
 ACCTTTGTCCCTTANTTGAAGGGTTAATTTCCAACCTTGGGGTT

16.

>call-254 = filamin
 ACTGAGGCGGGCCAGGGTAGATGTCAGCATTGGTATCAAGTGTGCCCTGGAGTACTGGG
 CCCCACTGAGGCTGATATTGACTTTTGATATCATCCGTAATGACAAATGACACCTTCACTGT
 GAAATACACACCCCTGTGGGCTGGCAGCTATACCATCATGTTCTTTTGTGCTGACCAGGC
 CACACCCACAGCCCATCACACTCAAATTGGAGCCTTCTCATGATGCCAGTAAAGTGAA
 TGCTGAGGGTCCCTGTCTAAATCGCACTGGTGTGAGCTTGGCAAAACCCACCCATTTCAC
 AGTCAATGCTAAAACTGCTGGGAAAGGCAAGCTGGATGTTCAAGTCTCACGACTGGCTAA
 CGGAGATTTC

FIG. 2A-9

17.
 >cal2_28 = Highly charged amino acid sequence (X59131)
 ACTAGGATAGAGCGGAGGCCCTAGGAGAGGAGAGAGCCACCCACAAGGCTCCCTGTCCACAG
 TGCACAGTCAGTTGTTGAGATTCCACTGTCTGCAGGGTCCCTTGTCTCAAAATACCTGTT
 AATAATATTACATTGAAATAATAGGTCCCCGAAGCCACTTTTGATATTCTAGGAGTCCG
 GGAGTATTTCAAAGCTAGGGCCATGCGGAGAGGGCCTTTGTTCAAGGATTTTAGTCTTAG
 GTCATCAGGCCCTCCAAACTTATCAGCCTTGAGCAACTCAACAAGACTCCTATCCACCCA
 NCCTGGNCCCTCCCCCACCCTTTCTACCGTCTCAGGCATGGGACACTGTGGGTTTC
 CTNAACCTGTGACTGATGCCCC
18.
 >cal2_204 = Kidney aminopeptidase M/kidney Zn-peptidase
 aminopeptidase N
 ACACGTAGTCCACTCCAGGCTCACAAAGCCTTGCTAGTCGTTTCATTCAGGCTGACCTGAT
 CCTGTGACTGATGCATGAAGCAGTTCTCCTCGGCACAGCGGCACATTTTCATTGTGGCACA
 GCTTGCTCAGCATTCATCGTCCTTCTCCGGATGATANAACCGGTGCATGACTCCTCTA
 GATTGTAGTAGGATAGACCTTGACCGACCCCGGTGGATAAGTCCCACGTTAAAGAACT
 GGTGGACTTTGAAGGACAGGCAGTCCTTCTTCGGAGTGTGAGATCTTTCTAGGTAGATGA
 TGAGGGTGTCTTGTGGAGAGGCTTTGTCCATCTCATACTTGGAAATGTATCTGTCTA
 CTCCAGAGCTCAGNNGTNCAGGTCGTTTGTGTCTGGAATAAAGCCANTCNTCATGGAGA
 TGTCCAGGATGGACATAGTAGCATCCNCGTCTCCCAAGT

FIG. 2A-10

19.

>call-268 = Myosin heavy chain A, non-muscle
 ACTCCTCCTGCTCCAGNATGAACATGGTGTGGTTGAACAGCTGCTGCAGC
 TTCTCGTNGGTNACTNGATNTCAAAGTTGCTCGAATGAGTTCACATCAA
 AGATCTCGAAGCCAGCGGATGTCCANGATCNCGANNTATGANGCNCCTG
 CCTCTTGGTCTTGTCCNNTAACTTTGTTATTGNGAACCAACCAGCCAGGGGA
 ATATCCGCNCGTAGGTACCCCTTNGNCAGAGCCCTCCATGGTAAATCAGCC
 TGCNCTTTCTGTGGCCNTCTGCNCATNCTCGCTGCCNTNCNTNATGCN
 CGGGTCAAGGATNCCTCTGGTTGACCTCGGTCNCNTTNCATCNCCTCAC
 GCAAGTNGGACANCTNTTGAACCTCCTGTGTTATCTGGCATGGACGACTT
 GTTCAGNTGCTCCCTCCCTNTNCTTGANNGACCCANGTNCCTCTCTGTC
 TNAATGTACCCCTTGANATGATCTCTCAG

20.

>call14d6t7 = Novel protein AHNAK (nucleoprotein) (M80899)
 ACATTTTCCAAACAATGGTTTCTTGACTTTGGGAGATTTTACTTCAGCTTTGAGCGCAG
 CATCAGGCCCCCTGCAACGCTGACATCCACATCAGGAACTTTGAGGCTGGCATCAATCTCAC
 CTCCAGNAACATTCAAATCAATCCCTGTTTCTTGGTCTTGACTTTAGGGCCACTCATGT
 GAATTTAGGCATTTTAAACTTGCTTTTCTTGCCCTTGCCACCAATGTTTATCTCAGGAG
 TCTCAACACTCAGTTCTGCTTCAGGGACAGTCACACCAACTGTGGGGTCTTAATGTCTAG
 CTTTGGGGCTTTTGCACCAAAATCCAAACTTGGGTTTAACTTCGGAACCTTAAACAT
 CTGGCCCTTCAATATAATATCTGGACTCTCCATGTGGACATCTAAGCTTGGAGTTTCTA
 AATCAACTTTAGGCCCCCTGAGGTCAACTTCACCTCCTCCTAACTTAGGCCCAAGAAATC
 CAACATTTGGTGTCTTGAGGTGCACATTAGCATCAGGCCCACTTATTTTAGGAACAGGT

FIG. 2A-11

21. >call-191 = Secretory protein containing thrombospondin motifs
 ACCAGACCTTGTCAGACCTTCCTTGCCACGTTGGCAGGTGGGGATTG
 GTCACCATGTTCTAAACTTGTGGGAAGGTTATAAGAAAGAGAACCTTGA
 AATGTCGTCCCATGATGGCGGTGTATCAAAATGAGAGCTGTGATCCT
 TTGAATAAACCAAGCATTACATTGACTTTTGCCATACTGACACAGTGCAG
 TTAACAGGTTTGGAGGACAAGGTAGCGTGGAGGGCTGATACACTGAAAG
 CAAGAGTGCTGGAGGGATCCTGTGAATCAAACCAgTAAACAGTGAGGTGT
 GGCAATGAGGTGTGTATAGGGGATaCATTAGCAAAGAGGTAGATCCGT
22. >call-562 = Stearoyl Co A desaturase
 ACTGTGCAGCGGGCTGGCTGCAGAAACTTAAGTCATAACACTCTTTGTCGCTGAGGCTG
 AAGCTCTCTGCACGTTCTCATCCCTGGGAACGTGACCCAGCATCCGACGCCAAGATGCC
 GGCTCACATACTGCAAGAGATCTCTGGCTCTTACTCGGCCACCAACACATCACAGCGCC
 ACCTTCTGGGGACAGCAGAAATGGAGGAGAGAGTTTGAAGGAATCTTCACCACTGGGG
 AGCAGATGTTCGCCCTGAAATTAAAGATGACCTATACGACCCAGCTACCAGGATGAGGA
 GGGGCCCCCGCCCAAGCTGGAGT

FIG. 2A-12

23.

```
>cal2_76 = Tenascin
CATGGAGCTCCACGGGTGGCGCCGCTCTAGAATAGTGGATCCCCGGGCTGCAGGA
ATTCGATACTCATGGGCCCTTCCAGTGGAAACCATTTAAAAACCCCTGAATGGTGAATA
ATGTTCCCATATCTGCCCATCAAGTTGAANCCATGANAGTTCTTATACCAGAAAGCTCC
TTTGTNGGACAGGGCACAGTTGGTGATGGCTGAATCNGTGTCTTGTCTATAGTNGANAA
NGATCTACCATTTGTGATAGTTTCATGGAATCACCTACCGTTCCTACTGTATCCTTCTACCTT
CAGCTGTAGCGACTCTTGGCATCTCCAACACTGAACCTGTCTATACACAGCATAAAGCTGA
CTCCCCATGGTCTTTGTAGGTCCACCCGGANCTCATATTGCCCTTGGGCTGTGATTTTCT
CAGGTTATCCAGTCCAAGCCAGAATTCTTCTCTGCGGTCTCCAAACCCCAATGGCATTAGC
CTTCCAGTTTCCAAAGAAANTCCTCACGTCCATTTTTCGCTCTCAAGAAACGANTCATC
CACCTCCATCC
```

24.

```
>cal1-609 = Thrombospondin 1
ACCACTGAAGTCCACGGCATTAACCTCATATAACCTACAGCAAGTCCAGGTCACAGTT
TACAGTCTGGACAAGTTCTTTGCCCTGATGGCGGACAACCCAGTTAGGGTCATTTTGGGA
GGTTCCTTTGGGATCTAGAGGAATCATCTGGAATCGCGGAAATCGGTTTCACTGATGTC
AACATTCTCAGGACAGATGTCATCAATGTCTGGCACATTGTCTGTTCAAAGTCGTCTTT
GCAGGCATCGCCTCGGCATCACCATCAGAGTCTTCTGTCAGGATGGGCACACAGCCT
GCAGTTGTCTCTGTATCAGGGATGCCGTGTTGTCTATCGTCTGTCACAGGCGTCTCC
TTTACCATCTTTATCGTGGTCGGCCTGGTTGGCATTGGGCACATAGGACAGTTGTCCAC
GTTGTTCTGATGGCCATCCTCATCGATGTCCTGATTGTTGTC
```

FIG. 2A-13

25.

>cal1-700 DEST
ACTCTCCCAGGTGTTGAGGTGGAGGAAAGGGGCTTCTGCACCTTCGATGATGGCCTCG
ATACCTGACTCGGGGTTCTGCAGCTCTGTTGGTAGCACTGCTGGCAGGAAACTTCCTG
CCTCCTGGATGAAGGAAGGTTTACTCATCATCACTGGCAGCTGCAGGAANTCGGGGCC
TGATAAACAAATTCATAACCCCTTTCTGCTAGTCCGCTGCCAAAAACTCAAAGGACCC
AAGCAGGGCAGCTGTTCCACAGGCGAGCCACTGTCACTGAGT

26.

>cal1-248 Connective tissue growth factor
ACTACAGGAAGATGTATGGAGACATGGCGTAAAGCCAGGGAGTAAGGGACACGAACTCAT
TTAGACTATAACTTGAACTGAGTTACATCTCATTTTCTGTAAAAAACAACAAAGGAT
TACAGTAGCACATTAATTTAAATCTGGGTTCCCTAACTGCTGTGGAGAAACACCCACCC
GAAGTGAGAACCGTGTGTCATTGTGTCATGCAATAGCCCTGTCAATCTCAGACACTGGTTTC
GAGACAGTTTAGACTTGACAGTTGTTCACTAGCGCACAGTGACAGAACGCACACTAAGGT
GAGCCTCCTGGCAAGAGTGGACATGCCANCGAGAAAGACAGGT

27.

>cal2-45 SARF1
ACTACAGCTATTCAAAACTACCCTTAAATGAGCTGTTTAAACTTCATAAAAAACAGT
CATGATTTTATTAGTTGTGAATATTTCTACAAGATCTGGATGGACTTTTCGCTTTATGTG
AAAATAGCTATCTGTTCCCGGGCCCTCCTAGGGCCTTATAACTCAGGACACGTTAGGATA
CACACATGCAAAAGGCAGGGCAAGCTGCTCCAAGCTGAGACTGTAGCTCTCCCCGACAGT

FIG. 2A-14

28. >call3c4 Syntrophin
ACACAGAAAGCCATTCCGAGGCTGGGACCTGACCCCTGTGGGATCCACCCCATCTCCAGTC
AACAGCAGAGCTGECACACACTCTGGAGGCCCTAAGCCACCTGAGATCTCTGCAGATG
GAGTCTTCTGGGTCAGTGGCAANAGGAGGAAGAGGAGCCTTTCTGTTCACTCTCCCCTGG
GCCCCGAGGGCTGTCTGGGCTCTCTCCTCCTCTCTCTCTCTCTCTCTTGATATAAACAGC
CTGTGAGCATAGACAAAAAATAAAAAAATAAAAAAAGCTTGT

29. >call-404 erg25 methyl sterol
ACTGGACATCCGTCCCAAAGATTCTGTCCCACACGTGAAGGTGGAGGCGTAATTCCCAA
TGAAGTTCATGTGGTGAATCGTGATCCGAGCCCCGGTATAAAAAGGATGTAGTTCA
ACGGGTTGAGAGGGATATCATAAACCACTGTGCACATCGATGGTTCTAGCAAAACGCATGG
TCACCCATGCCCACAGAAGATTACATGATCACACAAAAGCACAAATCCAATGAAAAAAT
CCAGTCCCAAGAATTAGGGTTTCCAAGGGATGTGCATATTCTGCTTCGATTCCAAATGGA
GCCTGGAAACTCGTGATGGACTTTATGAATGTATTTATAAATCCTCTTGTGGTGAAGGAG
TCTGTGCAGGAAATAGTGCCAGGTATCCTCGATGACTGCACAGCCCCAAACACCTTGCCAA
AGTGAAATACCATCTTGGCATTTCTTCCCAATCATAGGAATATTGAAAAAACTCTGTAAA
ATAGTATGTTCCACAAATCAGAAAGCAACTGGATATAAAAAAATGATTAACAGAAATTCCTTT
CAAAACACTTCACCTGGCCCTTCAAAGGTTCTGGTTTATCCTTTTGAATCTTGT

FIG. 2A-15

30.

>cal2-176 DEST
ACCATAAGCAGTTTCCATGGGGTCAGAGAATGGCTGGAGAGTTGTGGAACCCAGCAGGAG
GTATTGTTTGGAGACAGGACCTCACTGTGTAGCCCTGGCCTCAAACCTCAGAGATCCACC
TGCCCTCGCCTCCTGAGTGCTTGGATTAAAGGCATGCACCAACCGCCGCTGACAGGG
AGGAAGTTCTGATGGAGGAGCTATTGACAGGTAGCAGAGACAGCCGAAAGACGATCATG
GGCCATCTGTGGGTCAGTGGGGCTGCTGTATGCTTTTGCTGCTTTACAGGGAAGGTGA
TGTATGCATCGTAGCCAAACAGCAAGGTAGCCANTAAAGCCCAGT

31.

>OSTEONECTIN
ACTTGTCATTGTCTAGGTCACAGGTCTCAAAGAAGCGAGTGGTGCAATGTTCATGGGAA
TGAGGGGAGCGCGCAGTGGGGCCAGCTCCGTGTGGGACAGGT

*FIG. 3A***Protein Sequence of 608**

Length of 608 13.5.99: 8883 bp; Listed from: 2 to: 8883;
 Translated from: 575 to: 8365 (Entire region);
 Genetic Code used: Universal;

Frame 2

GAG	AGA	CGA	CAG	AAG	GTT	ACG	GCT	GCG	AGA	AGA	CGA	CAG	AAG	GGT	CCA	GAA	AAA	55	
			10		19		28		37		46								
CGA	AAG	TGC	TGG	AGG	GGA	GTG	GGG	ACA	AAA	GCA	GCG	ACC	AAG	TGA	ATG	TCA	CTT	CAG	TGA
		64			73		82		91		100					109			
CTG	AGG	CCA	GGC	AAA	ACG	CGC	GGG	AAG	GAT	TTT	GTG	TAG	CTT	GGG	ACC	CTT	TCA	TAG	ACA
		121			133		142		151		160					169			
CTG	ATG	ACA	CGT	TTA	CGC	AAA	ATA	GAA	ATT	TGA	GGA	ACG	CCT	GGG	CCT	TCG	GAA	AGG	
		184			193		202		211		220					229			

FIG.3B

AGT GAT TGA TTA GTA CTT GCA AGT TTA GGT GAC TTT AAG GAG AAC TAA CTA ATG TAT ACT	244	253	262	271	280	289
ATT GAG GGA GGA GGA AGA GCA TTA CAG AGT TTC CAG CAG CAG GAA AGC TTT GGT TAA	304	313	322	331	340	349
TTT GGA AAT GGA TGA TAG CAT TAA AAT AAC AGA AGC GCC TCC AGG TCT CTG AAG CTT CAG	364	373	382	391	400	409
TCC CCC AGC TGA AAG CCA GAA AAG ACT AAG CCC ACT AAG CCT TTT GAT CCC TTT GGA AGC	424	433	442	451	460	469
AAA GAA CTT TCC TTC CCT GGG GTG AAG ACT CTC AGA AGA TTT CCT GTC TCT GCC TAT	484	493	502	511	520	529

FIG. 3C

GTT	ACA	AGA	GGA	ATC	AAA	ACC	AAG	ACA	GAA	GAG	CTC	AGG	ATG	M	Q	V	R	G	R	E	
		544			553		562				571		580								
V	S	G	L	L	I	S	L	T	A	V	C	L	V	V	V	T	P	G	S	R	
GTC	AGC	GGC	TTG	TTG	ATC	TCC	CTC	ACT	GCT	GTC	TGC	CTG	GTG	GTC	ACC	CCT	GGG	AGC	AGG		
		604			613		622				631		640								
A	C	P	R	R	C	A	C	Y	V	P	T	E	V	H	C	T	F	R	Y		
GCC	TGT	CCT	CGC	CGC	TGT	GCC	TGC	TAT	GTG	CCC	ACA	GAG	GTG	CAC	TGT	ACA	TTT	CGG	TAC		
		664			673		682				691		700								
L	T	S	I	P	D	G	I	P	A	N	V	E	R	I	N	L	G	Y	N		
CTG	ACC	TCC	ATC	CCA	GAT	GGC	ATC	CCG	GCC	AAT	GTG	GAA	CGA	ATA	AAT	TTA	GGA	TAT	AAC		
		724			733		742				751		760								
S	L	T	R	L	T	E	N	D	F	D	G	L	S	K	L	E	L	L	M		
AGC	CTT	ACT	AGA	TTG	ACA	GAA	AAC	GAC	TTT	GAT	GGC	CTG	AGC	AAA	CTG	GAG	TTA	CTC	ATG		
		784			793		802				811		820								

FIG. 3D

L H S N G I H R V S D K K T F S G L Q S L
 CTG CAC AGT AAT GGC ATT CAC AGA GTC AGT GAC AAC ACC TTC TCG GGC TTG CAG TCC TTG
 844 853 862 871 880 889

Q V L K M S Y N K V Q I I R K D T F Y G
 CAG GTC TTA AAA ATG AGC TAT AAC AAA GTC CAA ATC ATT CGG AAG GAT ACT TTC TAC GGA
 904 913 922 931 940

L G S L V R L L H L D H N N I E F I N P E
 CTC GGG AGC TTG GTC CCG TTG CAC CTG GAT CAC AAC AAC AAC ATT GAA TTC ATC AAC CCT GAG
 964 973 982 991 1000 1009

A F Y G L T S L R L V H L E G N R L T K
 GCC TTT TAT GGA CTT ACC TCG CTC CGC TTG GTA CAT TTA GAA GGA AAC CGG CTC ACA AAG
 1024 1033 1042 1051 1060 1069

L H P D T F V S L S Y L Q I F K T S F I
 CTC CAT CCA GAC ACA TTT GTC TCA TTA AGC TAT CTC CAG ATA TTT AAA ACC TCT TTC ATT
 1084 1093 1102 1111 1120 1129

FIG.3E

K Y L F L S D N F L T S L P K E M V S Y
 AAG TAC CTG TTC TTG TCT GAT AAC TTC CTG ACC TCC CTC CCA AAA GAA ATG GTC TCC TAC
 1144 1153 1162 1171 1180 1189

 M P N L E S L Y L H G N P W T C D C H L
 ATG CCA AAC CTA GAA AGC CTG TAT TTG CAT GGA AAC CCA TGG ACC TGT GAC TGC CAT TTA
 1204 1213 1222 1231 1240 1249

 K W L S E W M Q G N P D I I K C K K D R
 AAG TGG TTG TCT GAG TGG ATG CAG GGA AAC CCA GAT ATA ATA AAA TGC AAG AAA GAC AGA
 1264 1273 1282 1291 1300 1309

 S S S S P Q Q C P L C M N P R I S K G R
 AGC TCT TCC AGT CCT CAG CAA TGT CCC CTT TGC ATG AAC CCC AGG ATC TCT AAA GGC AGA
 1324 1333 1342 1351 1360 1369

 P F A M V P S G A F L C T K P T I D F S
 CCC TTT GCT ATG GTA CCA TCT GGA GCT TTC CTA TGT ACA AAG CCA ACC ATT GAT CCA TCA
 1384 1393 1402 1411 1420 1429

FIG. 3F

L K S K S L V T Q E D N G S A S T S P 2
 CTG AAG TCA AAG AGC CTG GTT ACT CAG GAG GAC AAT GGA TCT GCC TCC ACC TCA CCT CAA
 1444 1453 1462 1471 1480 1489

 D F I E P F G S L S L N M T X S G N K
 GAT TTC ATA GAA CCC TTT GGC TCC TTG TCT TTT AAC ATG ACA KAS MTK TCT GGA AAT AAG
 1504 1513 1522 1531 1540 1549

 A D M V C S I Q K P S R T S P T A F T E
 GCC GAC ATG GTC TGT AGT ATC CAA AAG CCA TCA AGG ACA TCA CCA ACT GCA TTC ACT GAA
 1564 1573 1582 1591 1600 1609

 E N D GAC TAC ATC ATG CTA AAT GCG TCA TTT TCC ACA AAT CTT GTG TGC AGT GTA GAT
 1624 1633 1642 1651 1660 1669

 Y N H I Q P V W Q L L A L Y S D S P L I
 TAT AAT CAC ATC CAG CCA GTG TGG CAA CTT CTG GCT TTA TAC AGT GAC TCT CCT CTG ATA
 1684 1693 1702 1711 1720 1729

FIG. 3G

L E R K P Q CAG CTT ACC GAG ACT CCT TCA CTG TCT TCT AGA TAT AAA CAG GTG
 CTA GAA AGG AAG CCC CAG CTT ACC GAG ACT CCT TCA CTG TCT TCT AGA TAT AAA CAG GTG
 1744 1753 1762 1771 1780 1789

 A L R P E D I F T S I E A D V R A D P F
 GCT CTT AGG CCT GAA GAC ATT TTT ACC AGC ATA GAG GCT GAT GTC GCA GAC CCT TTT
 1804 1813 1822 1831 1840 1849

 W F Q Q E K I V L Q CAG CTG AAC AGA ACT GCC ACC ACA CTT AGC ACA
 TGG TTC CAA CAA GAA AAA ATT GTC TTG CAG CTG AAC AGA ACT GCC ACC ACA CTT AGC ACA
 1864 1873 1882 1891 1900 1909

 L Q I Q F S T D A Q I A L P R A E M R A
 TTA CAG ATC CAG TTT TCC ACT GAT GCT CAA ATC GCT TTA CCA AGG GCG GAG ATG AGA GCG
 1924 1933 1942 1951 1960 1969

 E R L K W T M I L M M N N P K L E R T V
 GAG AGA CTC AAA TGG ACC ATG ATC CTG ATG AAC AAT CCC AAA CTG GAA CGC ACT GTC
 1984 1993 2002 2011 2020 2029

FIG. 3H

L V G G G T I A L S C P G K G D P S P H L
 CTG GTT GGC GGC ACT ATT GCC CTG AGC TGT CCA GGC AAA GGC GAC CCT TCA CCT CAC TTG
 2044 2053 2062 2071 2080 2089

 E W L L A D G S K V R A P Y V S E D G R
 GAA TGG CTT CTA GCT GAT GGG AGT AAA GTG AGA GCC CCT TAC GTT AGC GAG GAT GGG CGA
 2104 2113 2122 2131 2140 2149

 I L I D K N G K L E L Q M A D S F D A G
 ATC CTA ATA GAC AAA AAT GGG AAG TTG GAA CTG CAG ATG GCT GAC AGC TTT GAT GCA GGT
 2164 2173 2182 2191 2200 2209

 L Y H C I S T N D A D A D V L L T Y R I T
 CTT TAC CAC TGC ATA AGC ACC AAT GAT GCA GAT GCG GAT GTT CTC ACA TAC AGG ATA ACT
 2224 2233 2242 2251 2260 2269

 V V E P Y G E S T H D S G V Q CAG CTC CAC ACA GTG GTT ACG
 GTG GTA GAG CCC TAT GGA GAA AGC ACA CAT GAC AGT GGA GTC CAG CAC ACA GTG GTT ACG
 2284 2293 2302 2311 2320 2329

G	E	T	L	D	L	P	C	L	S	T	G	V	P	D	A	S	I	S	TGG
GGT	GAG	ACG	CTC	GAC	CTT	CCA	TGC	CTT	TCC	ACG	GGT	GTT	CCA	GAT	GCT	TCT	ATT	AGC	2389
		2344			2353			2362		2371			2380						
I	L	P	G	N	T	V	F	S	Q	P	S	R	D	R	Q	I	L	N	N
ATT	CTT	CCA	GGG	AAC	ACT	GTG	TTC	TCT	CAG	CCA	TCA	AGA	GAC	AGG	CAA	ATT	CTT	AAC	AAT
		2404			2413			2422		2431			2440				2449		
G	T	L	R	I	L	Q	V	T	P	K	D	Q	G	H	Y	Q	C	V	A
GGG	ACC	TTA	AGA	ATA	TTA	CAG	GTT	ACG	CCA	AAA	GAT	CAA	GGT	CAT	TAC	CAA	TGT	GTG	GCT
		2464			2473			2482		2491			2500				2509		
A	N	P	S	G	A	D	F	S	S	F	K	V	S	V	Q	K	K	G	Q
GCC	AAC	CCA	TCA	GGG	GCC	GAC	TTT	TCC	AGT	TTT	AAA	GTT	TCA	GTT	CAA	AAG	AAA	GGC	CAA
		2524			2533			2542		2551			2560				2569		
R	M	V	E	H	D	R	E	A	G	G	S	G	L	G	E	P	N	S	S
AGG	ATG	GTT	GAG	CAT	GAC	AGG	GAG	GCA	GGT	GGA	TCT	GGA	CTT	GGA	GAA	CCC	AAC	TCC	AGT
		2584			2593			2602		2611			2620				2629		
V	S	L	K	Q	P	A	S	L	K	L	S	A	S	A	L	T	G	S	E
GTT	TCC	CTT	AAG	CAG	CCA	GCA	TCT	TTG	AAA	CTC	TCT	GCA	TCA	GCT	TTG	ACA	GGG	TCA	GAG
		2644			2653			2662		2671			2680				2689		

FIG. 3J

A G K Q V S G V H R K N K H R D L I H R
 GCT GGA AAA CAA GTC TCC GGT GTA CAT AGG AAG AAC AAA CAT AGA GAC TTA ATA CAT CGG
 2704 2713 2722 2731 2740 2749

 R R G D S T L R R F R E H R R Q L P L S
 CGG CGT GGG GAT TCC ACG CTC CGG CGA TTC AGG GAG CAT AGG AGG CAG CTC CCT CTC TCT
 2764 2773 2782 2791 2800 2809

 A R R I D P Q R W A A L L E K A K K N S
 GCT CGG AGA ATT GAC CCG CAA CGC TGG GCA GCA CTT CTA GAA AAA GCC AAA AAG AAT TCT
 2824 2833 2842 2851 2860 2869

 V P K K Q E N T T V K P V P L A V P L V
 GTG CCA AAA AAG CAA GAA AAT ACC ACA GTA AAG CCA GTG CCA CTG GCT GTT CCC CTC GTG
 2884 2893 2902 2911 2920 2929

 E L T D E E K D A S G M I P P D E E F M
 GAA CTC ACT GAC GAG GAA AAG GAT GCC TCT GGC ATG ATT CCT CCA GAT GAA GAA TTC ATG
 2944 2953 2962 2971 2980 2989

 V L K T K A S G V P G R S P T A D S G P
 GTT CTG AAA ACT AAG GCT TCT CCA GGA AGG TCA CCA ACT GCT GAC TCT GGA CCA
 3004 3013 3022 3031 3040 3049

FIG. 3K

V N H G F M T S I A S G T E V S T V N P
 GTA AAT CAT GGT TTT ATG ACG AGT ATA GCT TCT TGT GGC ACA GAA GTC TCA ACT GTG AAT CCA
 3064 3073 3082 3091 3100 3109

 Q T L Q S E H L P D F K L F S V T N G T
 CAA ACA CTA CAA TCT GAG CAC CTT CCT GAT TTC AAA TTA TTT AGT GTA ACA AAC GGT ACA
 3124 3133 3142 3151 3160 3169

 A V T K S M N P S I A S K I E D T T N Q
 GCT GTG ACA AAG AGT ATG AAC CCA TCC ATA GCA AGC AAA ATA GAA GAT ACA ACC AAC CAA
 3184 3193 3202 3211 3220 3229

 N P I I I F P S V A E I R D S A Q A G R
 AAC CCA ATC ATT ATC TTT CCA TCA GTA GCT GAA ATT CGA GAT TCT GCT CAG GCA GGA AGA
 3244 3253 3262 3271 3280 3289

 A S S Q S A H P V T G G N M A T Y G H T
 GCA TCT TCC CAA AGT GCA CAC CCT GTA ACA GGG GGA AAC ATG GCT ACC TAT GGC CAT ACC
 3304 3313 3322 3331 3340 3349

 N T Y S S F T S K A S T V L Q P I N P T
 AAC ACA TAT AGT AGC TTT ACC AGC AAA GCC AGT ACA GTC TTG CAG CCA ATA AAT CCA ACA
 3364 3373 3382 3391 3400 3409

FIG. 3L

E S Y G P Q I P I T G V S R P S S S D I
 GAA AGT TAT GGA CCT CAG ATA CCT ATT ACA GGA GTC AGC AGA CCT AGC AGT AGT GAC ATC
 3424 3433 3442 3451 3460 3469

 S S H T T A D P S F S S S H P S H T T
 TCT TCT CAC ACT ACT GCA GAC CCT AGC TTC TCC AGT CAC CCT TCA GGT TCA CAC ACC ACT
 3484 3493 3502 3511 3520 3529

 A S S L F H I P R N N N T G N F P L S R
 GCC TCG TCT TTA TTT CAC ATT CCT AGA AAC AAC AAT ACA GGT AAC TTC CCC TTG TCC AGG
 3544 3553 3562 3571 3580 3589

 H L G R E R T I W S R G R V K N P H R T
 CAC TTG GGA A GA GAG AGG ACA ATT TGG AGC AGA GGG AGA GTT AAA AAC CCA CAT AGA ACC
 3604 3613 3622 3631 3640 3649

 P V L R R H R H R T V R P A I K G P A N
 CCA GTT CTC CGA CGG CAT AGA CAC AGG ACT GTG AGG CCA GCA ATC AAG GGA CCT GCT AAC
 3664 3673 3682 3691 3700 3709

 K N V S Q V P A T E Y P G M C H T C P S
 AAA AAT GTG AGC CAA GTT CCA GCC ACA GAG TAC CCT GGG ATG TGC CAC ACA TGT CCT TCC
 3724 3733 3742 3751 3760 3769

FIG. 3M

A E G L T V A T A A L S V P S S H S A
 GCA GAG GGG CTC ACA GTG GCT ACT GCA GCA CTG TCA GTT CCA AGT TCA TCC CAC AGT GCC
 3784 3793 3802 3811 3820 3829

 L P K T N N V G V I A E E S T T V V K K
 CTC CCC AAA ACT AAT AAT GTT GGG GTC ATA GCA GAA GAG TCT ACC ACT GTG GTC AAG AAA
 3844 3853 3862 3871 3880 3889

 P L L L F K D K Q N V D I E I I T T T T
 CCA CTG TTA CTA TTT AAG GAC AAA CAA AAT GTA GAT ATT GAG ATA ATA ACA ACC ACT ACA
 3904 3913 3922 3931 3940 3949

 K Y S G G E S N H V I P T E A S M T S A
 AAA TAT TCC GGA GGG GAA AGT AAC CAC CAC GTG ATT CCT ACG GAA GCA AGC ATG ACT TCT GCT
 3964 3973 3982 3991 4000 4009

 P T S V S L G K S P V D N S G H L S M P
 CCA ACA TCT GTA TCC CTG GGG AAA TCT CCT CCT GTA GAC AAT AGT GGT CAC CTG AGC ATG CCT
 4024 4033 4042 4051 4060 4069

 G T I Q T G K D S V E T T P L P S L S
 GGG ACC ATC CAA ACT GGG AAA GAT TCA GTG GAA ACA CCA CTT CCC AGC CCC CTC AGC
 4084 4093 4102 4111 4120 4129

FIG. 3N

T P S I P T S T K F S K R K T P L H Q I
 ACA CCC TCA ATA CCA ACA AGC ACA AAA TTC TCA AAG AGG AAA ACT CCC TTG CAC CAG ATC
 4144 4153 4162 4171 4180 4189

 F V N N Q K K E G M L K N P Y Q F G L Q
 TTT GTA AAT AAC CAG AAG AAG GAG GGG ATG TTA AAG AAT CCA TAT CAA TTC GGT TTA CAA
 4204 4213 4222 4231 4240 4249

 K N P A A K K L P K I A P L L P T G Q S S
 AAG AAC CCA GCC GCA AAG CTT CCC AAA ATA GCT CCT CTT TTA CCC ACA GGT CAG AGT TCC
 4264 4273 4282 4291 4300 4309

 P S D S T T L L T S P P P A L S T T M A
 CCC TCA GAT TCT ACA ACT CTC TTG ACA AGT CCG CCA CCA GCT CTG TCT ACA ACA ATG GCT
 4324 4333 4342 4351 4360 4369

 A T Q N K G T E V V S G A R S L S A G K
 GCC ACT CAG AAC AAG GGC ACT GAA GTA GTA TCA GGT GCC AGA AGT CTC TCA GCA GGG AAG
 4384 4393 4402 4411 4420 4429

 K Q P F T N S S S P V L P S T I S K R S N
 AAG CAG CCC TTC ACC AAC TCC TCT CCA GTG CTT CCT AGC ACC ATA AGC AAG AGA TCT AAT
 4444 4453 4462 4471 4480 4489

FIG. 30

T L N F L S T E T P T V T S F T A T A S
ACA TTA AAC TTC TTG TCA ACG GAA ACC CCC ACA GTG ACA AGT CCT ACT GCT ACT GCA TCT
4504 4513 4522 4531 4540 4549

V I M S E T Q R T R S K E A K D Q I K G
GTC ATT ATG TCT GAA ACC CAA CGA ACA AGA TCC AAA GAA GCA AAA GAC CAA ATA AAG GGG
4564 4573 4582 4591 4600 4609

P R K N R N A N T T P R Q V S G Y S A
CCT CGG AAG AAC AGA AAC AAC GCA AAC ACC ACC CCC AGG CAG GTT TCT GGC TAT AGT GCA
4624 4633 4642 4651 4660 4669

Y S A L T T A A D T P L A F S H S P R Q D
TAC TCA GCT CTA ACA ACA GCT GAT ACC CCC TTG GCT TTC AGT CAT TCC CCA CGA CAA GAT
4684 4693 4702 4711 4720 4729

D G G N V S A V A Y H S T T S L L A I T
GAT GGT GGA AAT GTA AGT GCA GTT GCT TAT CAC TCA ACA ACC TCT CTT CTG GCC ATA ACT
4744 4753 4762 4771 4780 4789

E L F E K Y T Q T L G N T T A L E T T L
GAA CTG TTT GAG AAG TAC ACC CAG ACT TTG GGA AAT ACA ACA GCT TTG GAA ACA ACG TTG
4804 4813 4823 4831 4840 4849

FIG. 3P

L S K S Q E S T T V K R A S D T P P P L
 TTG AGC AAA TCA CAG GAG AGT ACC ACA GTG AAA AGA GCC TCA GAC ACA CCA CCA CTC
 4864 4873 4882 4891 4900 4909

 L S G A P P V P T P P P F T K G V
 CTC AGC AGT GGG GCG CCC CCA GTG CCC ACT CCT TCC CCA CCT CCT TTT ACT AAG GGT GTG
 4924 4933 4942 4951 4960 4969

 V T D S K V T S A F Q M T S N R V V T I
 GTT ACA GAC AGC AAA GTC ACA TCA CCT TTC CAG ATG ACG TCA AAT AGA GTG GTC ACC ATA
 4984 4993 5002 5011 5020 5029

 Y E S S R H N T D L Q Q Q P S A E A S P N
 TAT GAA TCT TCA AGG CAC AAT ACA GAT CTG CAG CAA CCC TCA GCA GAG GCT AGC CCC AAT
 5044 5053 5062 5071 5080 5089

 P E I I T G T T D S P S N L F P S T S V
 CCT GAG ATC ATA ACT GGA ACC ACT GAC TCT CCC TCT AAT CTG TTT CCA TCC ACT TCT GTG
 5104 5113 5122 5131 5140 5149

 P A L R V D K P Q N S K W K P S P W P E
 CCA GCA CTA AGG GTA GAT AAA CCA CAG AAT TCT AAA TGG AAG CCC TCT' CCC TGG CCA GAA
 5164 5173 5182 5191 5200 5209

FIG. 3Q

H K Y Q L K S Y S E T I E K G K R P A V
 CAC AAA TAT CAG CTC AAG TCA TAC TCC GAA ACC ATT GAG AAG GGC AAA AGG CCA GCA GTA
 5224 5233 5242 5251 5260 5269

 S M S P H L S L P E A S T H A S H W N T
 AGC ATG TCC CCC CAC CTC AGC CTT CCA GAG GCC AGC ACT CAT GCC TCA CAC TGG AAT ACA
 5284 5293 5302 5311 5320 5329

 Q K H A E K S V F D K K P G Q N P T S K
 CAG AAG CAT GCA GAA AAG AGT GTT TTT GAT AAG AAA CCT GGT CAA AAC CCA ACT TCC AAA
 5344 5353 5362 5371 5380 5389

 H L P Y V S L P K T L L K K P R I I G G
 CAT CTG CCT TAC GTC TCT CTA CCT AAG ACT CTA TTG AAA AAG CCA AGA ATA ATT GGA GGA
 5404 5413 5422 5431 5440 5449

 K A A S F T V P A N S D V F L P C E A V
 AAG GCT GCA AGC TTT ACA GTT CCA GCT AAT TCA GAC GTT TTT CTT CCT TGT GAG GCT GTT
 5464 5473 5482 5491 5500 5509

 G D P L P I I H W T R V S S G X E I S Q
 GGA GAC CCA CTG CCC ATC ATC CAC TGG ACC AGA GTT TCA TCA GGA NTT GAA ATA TCC CAA
 5524 5533 5542 5551 5560 5569

FIG. 3R

G T Q K S R F H V L P N G T L S I Q R V
 GGG ACA CAG AAA AGC CGG TTC CAC GTG CTT CCC AAT GGC ACC TTG TCC ATC CAG AGG GTC
 5584 5593 5602 5611 5620 5629

 S I Q D R G Q Y L C S A F N P L G V D H
 AGT ATT CAG GAC CGT GGA CAG TAC CTG TGC TCT GCA TTT AAT CCA CTG GGC GTA GAC CAT
 5644 5653 5662 5671 5680 5689

 F H V S L S V V F Y P A R I L D R H V K
 TTT CAT GTC TCT TTG TCT GTG GTT TTT TAC CCG GCA AGG ATT TTG GAC AGA CAT GTC AAG
 5704 5713 5722 5731 5740 5749

 E I T V H F G S T V E L K C R V E G M P
 GAG ATC ACA GTT CAC TTT GGA AGT ACT GTG GAA CTA AAG TGC AGA GTG GAG GGT ATG CCG
 5764 5773 5782 5791 5800 5809

 R P T V S W I L A N Q T V V S E T A K G
 AGG CCT ACG GTT TCC TGG ATA CTT GCA AAC CAA ACG GTG GTC TCA GAA ACG GCC AAG GGA
 5824 5833 5842 5851 5860 5869

 S R K V W V T P D G T L I I Y N L S L Y
 AGC AGA AAG GTC TGG GTA ACA CCT GAT GGA ACA TTG ATC ATC TAT TAT AGT CTT TAT
 5884 5893 5902 5911 5920 5929

FIG. 3S

D R G F Y K C V A S N P S G Q D S L L V
 GAT CGT GGT TTT TAC AAG TGT GTG GCC AGC AAC CCA TCT GGC CAG GAT TCA CTG TTG GTT
 5944 5953 5962 5971 5980 5989

K I Q V I T A P P V I I E Q K R Q A I V
 AAG ATA CAA GTC ATC ACA GCT CCC CCT GTC ATT ATA GAG CAA AAG AGG CAA GCC ATC GTT
 6004 6013 6022 6031 6040 6049

G V L G G S L K L P C T A K G T P Q P S
 GGG GTT TTA GGT GGA AGT TTG AAA CTG CCC TGC ACT GCA AAA GGA ACT CCC CAG CCT AGT
 6064 6073 6082 6091 6100 6109

V H W V L Y D G T E L K P L Q L T H S R
 GTT CAC TGG GTC CTT TAT GAT GGG ACT GAA CTA AAA CCA TTG CAG TTG ACT CAT TCC AGA
 6124 6133 6142 6151 6160 6169

F F L Y P N G T L Y I R S I A P S V R G
 TTT TTC TTG TAT CCA AAT GGA ACT CTG TAT ATA AGA AGC ATC GCT CCT TCA GTG AGG GGC
 6184 6193 6202 6211 6220 6229

T Y E C I A T S S S G S E R R V V I L T
 ACT TAT GAG TGC ATT GCC ACC AGC TCC TCA GCC TCA GAG AGA AGG GTA GTG ATT CTT ACT
 6244 6253 6262 6271 6280 6289

SUBSTITUTE SHEET (RULE 26)

FIG. 3U

K D F Q V D C K A S G S P V P E V S W S
 AAA GAT TTC CAA GTT GAC TGC AAG GCC TCT GGC TCC CCT GTG CCT GAG GTA TCC TGG AGT
 6664 6673 6682 6691 6700 6709

L P D G G T V L N N V A Q A D D S G Y R T
 TTG CCT GAT GGG ACA GTG CTC AAC AAT GTA GCC CAA GCT GAT GAC AGT GGC TAT AGG ACC
 6724 6733 6742 6751 6760 6769

K R Y T L F H N G T L Y F N N V G M A E
 AAG AGG TAC ACC CTT TTC CAC AAT GGA ACC TTG TAT TTC AAC AAC GTT GGG ATG GCA GAG
 6784 6793 6802 6811 6820 6829

E G D Y I C S A Q N T L G K D E M K V H
 GAA GGA GAT TAT ATC TGC TCT GCC CAG AAC ACC TTA GGG AAA GAT GAG ATG AAA GTC CAC
 6844 6853 6862 6871 6880 6889

L T V L T A I P R I R Q S Y K T T M R L
 CTA ACA GTT CTA ACA GCC ATC CCA CGG ATA AGG CAA AGC TAC AAG ACC ACC ATG AGG CTC
 6904 6913 6922 6931 6940 6949

R A G E T A V L D C E V T G E P K P N V
 AGG GCT GGA GAA ACA GCT GTT GTC CTT GAC TGC GAG GTC ACT GGG GAA CCG AAG CCC AAT GTA
 6964 6973 6982 6991 7000 7009

FIG. 3V

F W L L P S N N V I S F S N D R F T F H
 TTT TGG CTG CCT TCC AAC AAT GTC ATT TCA TTC TCC AAT GAC AGG TTC ACA TTT CAT
 7024 7033 7042 7051 7060 7069

A N R T L S I H K V K P L D S G D Y V C
 GCC AAT AGA ACT TTG TCC ATC CAT AAA GTG AAA CCA CTT GAC TCT GGG GAC TAT GTG TGC
 7084 7093 7102 7111 7120 7129

V A Q N P S G D D T K T Y K L D I V S K
 GTA GCT CAG AAT CCT AGT GGC CTG TAT GCA AAC ACT AAG ACA TAC AAA CTG GAC ATT GTC TCT AAA
 7144 7153 7162 7171 7180 7189

P P L I N G L Y A N K T V I K A T A I R
 CCT CCA TTA ATC AAT GGC CTG TAT GCA AAC AAG ACT GTT ATT AAA GCC ACA GCC ATT CGG
 7204 7213 7222 7231 7240 7249

H S K K Y F D C R A D G I P S S Q V T W
 CAC TCC AAA TAC TTT GAC TGC AGA GCA GAT GGG ATC CCA TCT TCC CAG GTC ACG TGG
 7264 7273 7282 7291 7300 7309

I M P G N I F L P A P Y F G S R V T V H
 ATT ATG CCA GGC AAT ATT TTC CTC CCA GCT CCA TAC TTT GGA AGC AGA GTC ACG GTC CAT
 7324 7333 7342 7351 7360 7369

FIG. 3W

P N G T L E M R N I R L S D S A D F T C
 CCA AAT GGA ACC TTG GAG ATG AGG AAC ATC CGG CTT TCT GAC TCT GCG GAC TTC ACC TGT
 7384 7393 7402 7411 7420 7429

V V R S E G G E S V L V V Q L E V L E M
 GTG GTT CGG AGC GAG GGA GGA GAG AGT GTG TTG GTA GTG CAG TTA GAA GTC CTA GAA ATG
 7444 7453 7462 7471 7480 7489

L R R P T F R N P F N E K V I A Q A G K
 CTG AGA AGA CCA ACA TTC AGA AAC CCA TTC AAC GAA AAA GTC ATC GCC CAA GCT GGC AAG
 7504 7513 7522 7531 7540 7549

P V A L N C S V D G N P P P E I T W I L
 CCC GTA GCA CTG AAC TGC TCT GTG GAT GGG AAC CCC CCA CCT GAA ATT ACC TGG ATC TTA
 7564 7573 7582 7591 7600 7609

P D G T Q F A N R P H N S P Y L M A G N
 CCT GAC GGC ACA CAG TTT GCT AAC AGA CCA CAC AAT TCC CCG TAT CTG ATG GCA GGC AAT
 7624 7633 7642 7651 7660 7669

G S L I L Y K A T R N K S G K Y R C A A
 GGC TCT CTC ATC CTT TAC AAA GCA ACT CGG AAC AAG TCA GGG AAG TAT CGC TGT GCA GCC
 7684 7693 7702 7711 7720 7729

FIG. 3X

R N K V G Y I E K L I L L E I G Q K P V
 AGG RAT AAG GTT GGC TAC ATC GAG AAA CTC ATC CTG TTA GAG AIT GGG CAG AAG CCA GTC
 7744 7753 7762 7771 7780 7789

 I L T Y E P G M V K S V S G E P L S L H
 ATT CTG ACA TAC GAA CCA GGG ATG GTG AAG AGC AGC GTC AGT GGG GRA CCG TTA TCA CTG CAT
 7804 7813 7822 7831 7840 7849

 C V S D G I P K P N V K W T T P G G H V
 TGT GTG TCT GAT GGC ATC CCC AAG CCA AAT GTC AAG TGG ACT ACA CCG GGT GGC CAT GTA
 7864 7873 7882 7891 7900 7909

 I D R P Q V D G K Y I L H E N G T L V I
 ATC GAC AGG CCT CAA GTG GAT GGA AAA TAC ATA CTG CAT GAA AAT GGC ACG CTG GTC ATC
 7924 7933 7942 7951 7960 7969

 K A T T A H D Q G N Y I C R A Q N S V G
 AAA GCA ACA GCT CAC GAC CAA GGA RAT TAT ATC TGT AGG GCT CAA AAC AGT GTT GGC
 7984 7993 8002 8011 8020 8029

 Q A V I S V S V M V V A Y P P R I I N Y
 CAG GCA GTT ATT AGC GTG TCA GTG ATG GTT GTG GCC TAC CCT CCC CGA ATC ATA AAC TAC
 8044 8053 8062 8071 8080 8089

FIG. 3Y

L P R N M L R R T G E A M Q L H C V A L
 CTA CCC AGG AAC ATG CTC AGG AGG ACA GGG GAA GCC ATG CAG CTC CAC TGT GTG GCC TTG
 8104 8113 8122 8131 8140 8149

 G I P K K P K V T W E T P R H S L L S K A
 GGA ATC CCC AAG CCA AAA GTC ACC TGG GAG ACG CCA AGA CAC TCC CTG CTC TCA AAA GCA
 8164 8173 8182 8191 8200 8209

 T A R K K P H R S E M L H F Q G T L V I Q
 ACA GCA AGA AAA CCC CAT AGA AGT GAG ATG CTT CAC CCA CAA GGT ACG CTG GTC ATT CAG
 8224 8233 8242 8251 8260 8269

 N L Q T S D S G V Y K C R A Q N L L G T
 AAT CTC CAA ACC TCG GAT TCC GGA GTC TAT AAG TGC AGA GCT CAG AAC CTA CTT GGG ACT
 8284 8293 8302 8311 8320 8329

 D Y A T T Y I Q V L
 GAT TAC GCA ACA ACT TAC ATC CAG GTA CTC TGA CAG GAA GGG GGA GAC TAA AAT TCA ACA
 8344 8353 8362 8371 8380 8389

FIG. 3Z

GAA GTC CAC ATC CAC AGG GTT TAT TTT TTG GAA GAA GTT TAA TCA AAG GCA GCC ATA GGC
 8404 8413 8422 8431 8440 8449

 ATG TAA ATG AGT CTG AAT ACA TTT ACA GTA TTA AAT TTA CAA TGG ACA TGC GAT GAG ACT
 8464 8473 8482 8491 8500 8509

 TGT AAA TGA AAG CAT TGT GAA CTG AAA CCG AGT CTC TGT GGA TCT CAA AGC AAA CTC TTA
 8524 8533 8542 8551 8560 8569

 ACT TAA GGC ACT TTG ATT TTG CCA ACA AAT AAT AAC AAA CAT TAA GAG AAA AAA ATG ATC
 8584 8593 8602 8611 8620 8629

 CAC TAC GAA ATA ACA AAC GGC TAA TGC ACC TGA ATT CTC AGT AAA AAG ACC TTT CTC TCG
 8644 8653 8662 8671 8680 8689

FIG. 3AA

CTA ACA GTT GCC AGC TGC CTC GTG TCT GTT TCC TAC CAA TGT CAC AAA CAT CGC ACA CAG
8704 8713 8722 8731 8740 8749

GGT GAA TGG AGT CAA CGG GAA AGA TTA AGT TTG CGG TCT GTG TAA ATC TCA ATG TAC AAA
8764 8773 8782 8791 8800 8809

TAT TCT GTC NCT GGT TTA TAA ACA TTT TGA TAA AAC CGA AAA AAA AAA AAA AAA AAA
8824 8833 8842 8851 8860 8869

AAA AAA AA

608-Rat

PROBE: 5' fragment of 608(Rat)

Target: (mRNA)

N-normal

T-Mechanical force

N T

28S

18S

FIG. 4

FIG. 5A

Length of 608 7680 bp; Listed from: 2 to: 7680;
 Translated from: 71 to: 7162 (Entire region);
 Genetic Code used: Universal;

Frame 2	L	E	S	L	Y	L	M	G	N	P	W	T	C	D	C	H	L	K
	CTA	GAA	AGC	CTG	TAT	TTG	CAT	GGA	AAC	CCA	TGG	ACC	TGT	GAC	TGC	CAT	TTA	AAG
	10			19				28				37			46			55
W	L	S	E	W	M	Q	G	N	P	D	I	I	K	C	K	K	D	R
	TGG	TTG	TCT	GAG	TGG	ATG	CAG	GGA	AAC	CCA	GAT	ATA	ATA	AAA	TGC	AAG	AAA	GAC
	64			73				82			91			100			109	AGC
S	S	S	P	Q	Q	C	P	L	C	M	N	P	R	I	S	K	G	R
	TCT	TCC	AGT	CCT	CAG	CAA	TGT	CCC	CTT	TGC	ATG	AAC	CCC	AGG	ATC	TCT	AAA	GGC
	124					133		142			151			160			169	CCC
F	A	M	V	P	S	G	A	F	L	C	T	K	P	T	I	D	P	S
	TTT	GCT	ATG	GTA	CCA	TCT	GGA	GCT	TTC	CTA	TGT	ACA	AAG	CCA	ACC	ATT	GAT	CCA
	184					193		202			211			220			229	TCA
																		CTG

FIG. 5B

K	S	K	S	L	V	T	Q	E	D	N	G	S	A	S	T	S	P	Q	D
AAG	TCA	AAG	AGC	CTG	GTT	ACT	CAG	GAG	GAC	AAT	GGA	TCT	GCC	TCC	ACC	TCA	CCT	CAA	GAT
		244			253			262			271			280			289		
F	I	E	P	F	G	S	L	S	L	N	M	T	D	L	S	G	N	K	A
TTC	ATA	GAA	CCC	TTT	GGC	TCC	TTG	TCT	TTG	AAC	ATG	ACA	GAC	CTG	TCT	GGA	AAT	AAG	GCC
		304			313			322			331			340			349		
D	M	V	C	S	I	Q	K	P	S	R	T	S	P	T	A	F	T	E	E
GAC	ATG	GTC	TGT	AGT	ATC	CAA	AAG	CCA	TCA	AGG	ACA	TCA	CCA	ACT	GCA	TTC	ACT	GAA	GAA
		364			373			382			391			400			409		
N	D	Y	I	M	L	N	A	S	F	S	T	N	L	V	C	S	V	D	Y
AAT	GAC	TAC	ATC	ATG	CTA	AAT	GCG	TCA	TTT	TCC	ACA	AAT	CIT	GTG	TGC	AGT	GTA	GAT	TAT
		424			433			442			451			460			469		
N	H	I	Q	P	V	W	Q	L	L	A	L	Y	S	D	S	P	L	I	L
AAT	CAC	ATC	CAG	CCA	CTG	TGG	CAA	CTT	CTG	GCT	TTA	TAC	AGT	GAC	TCT	CCT	CTG	ATA	CTA
		484			493			502			511			520			529		

E	R	K	AAG	CCC	P	Q	L	T	E	T	P	S	L	S	R	Y	K	Q	V	A
GAA	AGG	AGG	AAA	CTT	ACC	GAG	ACT	CCT	TCA	CTG	TCT	TCT	AGA	TAT	AAA	CAG	GTG	GCT		
			544	553			562						571		580		589			
L	R	P	E	D	I	F	T	S	I	E	A	D	V	R	A	GCA	GAC	CCT	TTT	
CTT	AGG	CCT	GAA	GAC	ATT	TTT	ACC	AGC	ATA	GAG	GCT	GAT	GTC	AGA	GCA	GAC	649		W	
			604		613			622			631			640					TGG	
F	Q	Q	E	K	I	V	L	Q	L	N	R	T	A	T	ACA	CTT	AGC	ACA	TTA	
TTC	CAA	CAA	GAA	AAA	ATT	GTC	TTG	CAG	CTG	AAC	AGA	ACT	GCC	ACC	700		709			
			664		673			682			691									
Q	I	Q	F	S	T	D	A	Q	I	A	L	P	R	A	GCG	GAG	ATG	AGA	GCG	
CAG	ATC	CAG	TTT	TCC	ACT	GAT	GCT	CAA	ATC	GCT	TTA	CCA	AGG	AGG	760		769		E	
			724		733			742			751									
R	L	K	W	T	M	I	L	M	M	N	N	P	K	L	E	R	T	V	L	
AGA	CTC	AAA	TGG	ACC	ATG	ATC	CTG	ATG	ATG	AAC	AAT	CCC	AAA	CTG	GAA	CGC	ACT	GTC	CTG	
			784		793			802			811			820			829			

FIG. 5D

V G G T I A L S C P G K G A A GGC AAA 871
 GTT GGC GGC ACT ATT GCC CTG AGC TGT CCA GGC 862
 844 853 862 871 880 889
 P H L E
 S P C C T T C A C C T T T G G A A

W L L A D G S K V R A P Y V S E D G R I
 TGG CTT CTA GCT GAT GGG AGT AAA GTG AGA GCC CCT CTT TAC GTT AGC GAG GAT GGC CGA ATC
 904 913 922 931 940 949
 L I D K N G K L E L Q M A D S F D A G L
 CTA ATA GAC AAA AAT GGG AAG TTG GAA CTG CAG ATG GCT GAC AGC TTT GAT GCA GGT CTT
 964 973 982 991 1000 1009

Y H C I S T N D A D A D V L T Y R I T V
 TAC CAC TGC ATA AGC ACC AAT GAT GCA GAT GCG GAT GTT CTC ACA TAC AGG ATA ACT GTG
 1024 1033 1042 1051 1060 1069

V E P Y C E S T H D S G V Q H T V V T G
 GTA GAG CCC TAT GGA GAA AGC ACA CAT GAC AGT GGA GTC CAG CAC ACA GTG GTT ACG GGT
 1084 1093 1102 1111 1120 1129

FIG. 5E

E	T	L	D	L	P	C	L	S	T	G	V	P	D	A	S	I	S	W	I
GAG	ACG	CTC	GAC	CTT	CCA	TGC	CTT	TCC	ACG	GGT	GTT	CCA	GAT	GCT	TCT	ATT	AGC	TGG	ATT
	1144				1153			1162			1171			1180			1189		
L	P	G	N	T	V	F	S	Q	P	S	R	D	R	Q	I	L	N	N	G
CTT	CCA	GGG	AAC	ACT	GTG	TTC	TCT	CAG	CCA	TCA	AGA	GAC	AGG	CAA	ATT	CTT	AAC	AAT	GGG
	1204				1213			1222			1231			1240			1249		
T	L	R	I	L	Q	V	T	P	K	D	Q	G	H	Y	Q	C	V	A	A
ACC	TTA	AGA	ATA	TTA	CAG	GTT	ACG	CCA	AAA	GAT	CAA	GGT	CAT	TAC	CAA	TGT	GTG	GCT	GCC
	1264				1273			1282			1291			1300			1309		
N	P	S	G	A	D	F	S	S	F	K	V	S	V	Q	K	K	G	Q	R
AAC	CCA	TCA	GGG	GCC	GAC	TTT	TCC	AGT	TTT	AAA	GTT	TCA	GTT	CAA	AAG	AAA	GGC	CAA	AGG
	1324				1333			1342			1351			1360			1369		
M	V	E	H	D	R	E	A	G	G	S	G	L	G	E	P	N	S	S	V
ATG	GTT	GAG	CAT	GAC	AGG	GAG	GCA	GGT	GGA	TCT	GGA	CTT	GGA	GAA	CCC	AAC	TCC	AGT	GTT
	1384				1393			1402			1411			1420			1429		

FIG. 5F

S L K Q P A S L K L S A S A L T G S E A
 TCC CTT AAG CAG CCA GCA TCT TTG AAA CTC TCT GCA TCA GCT TTG ACA GGG TCA GAG GCT
 1444 1453 1462 1471 1480 1489

 G K Q V S G V H R K N K H R D L I H R R
 GGA AAA CAA GTC TCC GGT GTA CAT AGG AAG AAC AAA CAT AGA GAC TTA ATA CAT CGG CGG
 1504 1513 1522 1531 1540 1549

 R G D S T L R R F R E H R R Q L P L S A
 CGT GGG GAT TCC ACG CTC CGG CGA TTC AGG GAG CAT AGG AGG CAG CTC CCT CTC TCT GCT
 1564 1573 1582 1591 1600 1609

 R R I D P Q R W A A L L E K A K K N S V
 CGG AGA ATT GAC CCG CAA CGC TGG GCA GCA CTT CTA GAA AAA GCC AAA AAG AAT TCT GTG
 1624 1633 1642 1651 1660 1669

 P K K Q E N T T V K P V P L A V P L V E
 CCA AAA AAG CAA GAA AAT ACC ACA GTA AAG CCA GTG CCA CTG GCT GTT CCC CTC GTG GAA
 1684 1693 1702 1711 1720 1729

FIG. 5G

L	T	D	E	E	K	A	S	G	M	I	P	P	D	E	E	F	M	V	
CTC	ACT	GAC	GAG	GAA	AAG	GAT	GCC	TCT	GGC	ATG	ATT	CCT	CCA	GAT	GAA	GAA	TTC	ATG	GTT
	1744				1753				1762		1771			1780				1789	
L	K	T	K	A	S	G	V	P	G	R	S	P	T	A	D	S	G	P	V
CTG	AAA	ACT	AAG	GCT	TCT	GGT	GTC	CCA	GGA	AGG	TCA	CCA	ACT	GCT	GAC	TCT	GGA	CCA	GTA
	1804				1813				1822		1831			1840				1849	
N	H	G	F	M	T	S	I	A	S	G	T	E	V	S	T	V	N	P	Q
AAT	CAT	GGT	TTT	ATG	ACG	AGT	ATA	GCT	TCT	GGC	ACA	GAA	GTC	TCA	ACT	GTG	AAT	CCA	CAA
	1864				1873				1882		1891			1900				1909	
T	L	Q	S	E	H	L	P	D	F	K	L	F	S	V	T	N	G	T	A
ACA	CTA	CAA	TCT	GAG	CAC	CTT	CCT	GAT	TTC	AAA	TTA	TTT	AGT	GTA	ACA	AAC	GGT	ACA	GCT
	1924				1933				1942		1951			1960				1969	
V	T	K	S	M	N	P	S	I	A	S	K	I	E	D	T	T	N	Q	N
GTG	ACA	AAG	AGT	ATG	AAC	CCA	TCC	ATA	GCA	AGC	AAA	ATA	GAA	GAT	ACA	ACC	AAC	CAA	AAC
	1984				1993				2002		2011			2020				2029	

FIG. 5H

P I I I F P S V A E I R D S A Q A G R A
 CCA ATC ATT ATC TTT CCA TCA GTA GCT GAA ATT CGA GAT TCT GCT CAG GCA GGA AGA GCA
 2044 2053 2062 2071 2080 2089

 S S Q S A H P V T G G N M A T Y G H T N
 TCT TCC CAA AGT GCA CAC CCT GTA ACA GGG GGA AAC ATG GCT ACC TAT GGC CAT ACC AAC
 2104 2113 2122 2131 2140 2149

 T Y S S F T S K A S T V L Q P I N P T E
 ACA TAT AGT AGC TTT ACC AGC AAA GCC AGT ACA GTC TTG CAG CCA ATA AAT CCA ACA GAA
 2164 2173 2182 2191 2200 2209

 S Y G P Q I P I T G V S R P S S D I S
 AGT TAT GGA CCT CAG ATA CCT ATT ACA GGA GTC AGC AGA CCT AGC AGT AGT GAC ATC TCT
 2224 2233 2242 2251 2260 2269

 S H T T A D P S S F S S H P S G S H T T A
 TCT CAC ACT ACT GCA GAC CCT AGC TTC TCC AGT CAC CCT TCA GGT TCA CAC ACC ACT GCC
 2284 2293 2302 2311 2320 2329

FIG. 5I

S	S	L	F	H	I	P	R	N	N	T	G	N	F	P	L	S	R	H	
TCG	TCT	TTA	TTT	CAC	ATT	CCT	AGA	AAC	AAC	AAT	ACA	GGT	AAC	TTC	CCC	TTG	TCC	AGG	CAC
	2344				2353			2362		2371			2380					2389	
L	G	R	E	R	T	I	W	S	R	G	R	V	K	N	P	H	R	T	P
TTG	GGA	AGA	GAG	AGG	ACA	ATT	TGG	AGC	AGA	GGG	AGA	GTT	AAA	AAC	CCA	CAT	AGA	ACC	CCA
	2404				2413			2422		2431			2440					2449	
V	L	R	R	H	R	H	R	T	V	R	P	A	I	K	G	P	A	N	K
GTT	CTC	CGA	CGG	CAT	AGA	CAC	AGG	ACT	GTG	AGG	CCA	GCA	ATC	AAG	GGA	CCT	GCT	AAC	AAA
	2464				2473			2482		2491			2500					2509	
N	V	S	Q	V	P	A	T	E	Y	P	G	M	C	H	T	C	P	S	A
AAT	GTG	AGC	CAA	GTT	CCA	GCC	ACA	GAG	TAC	CCT	GGG	ATG	TGC	CAC	ACA	TGT	CCT	TCC	GCA
	2524				2533			2542		2551			2560					2569	
E	G	L	T	V	A	T	A	A	L	S	V	P	S	S	S	H	S	A	L
GAG	GGG	CTC	ACA	GTG	GCT	ACT	GCA	GCA	CTG	TCA	GTT	CCA	AGT	TCA	TCC	CAC	AGT	GCC	CTC
	2584				2593			2602		2611			2620					2629	

FIG. 5J

P K T N N V G V I A E E S T T V V K K P
 CCC AAA ACT AAT AAT GTT GGG GTC ATA GCA GAA GAG TCT ACC ACT GTG GTC AAG AAA CCA
 2644 2653 2662 2671 2680 2689

 L L L F K D K Q N V D I E I I T T T K
 CTG TTA CTA TTT AAG GAC AAA CAA AAT GTA GAT ATT GAG ATA ATA ACA ACC ACT ACA AAA
 2704 2713 2722 2731 2740 2749

 Y S G G E S N H V I P T E A S M T S A P
 TAT TCC GGA GGG GAA AGT AAC CAC GTG ATT CCT ACG GAA GCA AGC ATG ACT TCT GCT CCA
 2764 2773 2782 2791 2800 2809

 T S V S L G K S P V D N S G H L S M P G
 ACA TCT GTA TCC CTG GGG AAA TCT CCT GTA GAC AAT AGT GGT CAC CTG AGC ATG CCT GGG
 2824 2833 2842 2851 2860 2869

 T I Q T G K D S V E T T P L P S P L S T
 ACC ATC CAA ACT GGG AAA GAT TCA GTG GAA ACA ACA CCA CTT CCC AGC CCC CTC AGC ACA
 2884 2893 2902 2911 2920 2929

FIG. 5K

P S I P T S T K F S K R K T P L H Q I F
 CCC TCA ATA CCA ACA AGC ACA AAA TTC TCA AAG AGG AAA ACT CCC TTG CAC CAG ATC TTT
 2944 2953 2962 2971 2980 2989

V N N Q K K E G M L K N P Y Q F G L Q K
 GTA AAT AAC CAG AAG AAG GAG GGG ATG TTA AAG AAT CCA TAT CAA TTC GGT TTA CAA AAG
 3004 3013 3022 3031 3040 3049

N P A A K L P K I A P L L P T G Q S S P
 AAC CCA GCC GCA AAG CTT CCC AAA ATA GCT CCT CTT TTA CCC ACA GGT CAG AGT TCC CCC
 3064 3073 3082 3091 3100 3109

S D S T T L L T S P P P A L S T T M A A
 TCA GAT TCT ACA ACT CTC TTG ACA AGT CCG CCA CCA GCT CTG TCT ACA ACA ATG GCT GCC
 3124 3133 3142 3151 3160 3169

T Q N K G T E V V S G A R S L S A G K K
 ACT CAG AAC AAG GGC ACT GAA GTA GTA TCA GGT GCC AGA AGT CTC TCA GCA GGG AAG AAG
 3184 3193 3202 3211 3220 3229

FIG. 5L

Q P F T N S S P V L P S T I S K R S N T
 CAG CCC TTC ACC AAC TCC TCT CCA GTG CTT CCT AGC ACC ATA AGC AAG AGA TCT AAT ACA
 3244 3253 3262 3271 3280 3289

 L N F L S T E T P T V T S P T A T A S V
 TTA AAC TTC TTG TCA ACG GAA ACC CCC ACA GTG ACA AGT CCT ACT GCT ACT GCA TCT GTC
 3304 3313 3322 3331 3340 3349

 I M S E T Q R T R S K E A K D Q I K G P
 ATT ATG TCT GAA ACC CAA CGA ACA AGA TCC AAA GAA GCA AAA GAC CAA ATA AAG GGG CCT
 3354 3373 3382 3391 3400 3409

 R K N R N A N T T P R Q V S G Y S A Y
 CGG AAG AAC AGA AAC AAC GCA AAC ACC ACC CCC AGG CAG GTT TCT TAT AGT GCA TAC
 3424 3433 3442 3451 3460 3469

 S A L T A D T P L A F S H S P R Q D D
 TCA GCT CTA ACA ACA GCT GAT ACC CCC TTG GCT TTC AGT CAT TCC CCA CGA CAA GAT GAT
 3484 3493 3502 3511 3520 3529

FIG. 5M

G G N V S A V A Y H S T T S L L A I T E
 GGT GGA AAT GTA AGT GCA GTT GCT TAT CAC TCA ACA ACC TCT CTT CTG GCC ATA ACT GAA
 3544 3553 3562 3571 3580 3589

 L F E K Y T Q T L G N T T A L E T T L L
 CTG TTT GAG AAG TAC ACC CAG ACT TTG GGA AAT ACA GCT TTG GAA ACA ACG TTG TTG
 3604 3613 3622 3631 3640 3649

 S K S Q E S T T V K R A S D T P P P L L
 AGC AAA TCA CAG GAG AGT ACC ACA GTG AAA AGA GCC TCA GAC ACA CCA CCA CTC CTC
 3664 3673 3682 3691 3700 3709

 S S G A P P V P T P S P P P P F T K G V V
 AGC AGT GGG GCG CCC CCA GTG CCC ACT CCT TCC CCA CCT CCT TTT ACT AAG GGT GTG GTT
 3724 3733 3742 3751 3760 3769

 T D S K V T S A F Q M T S N R V V T I Y
 ACA GAC AGC AAA GTC ACA TCA GCT TTC CAG ATG ACG TCA AAT AGA GTG GTC ACC ATA TAT
 3784 3793 3802 3811 3820 3829

FIG. 5N

E S S R H N T D L Q Q P S A E A S P N P
 GAA TCT TCA AGG CAC AAT ACA GAT CTG CAG CAA CCC TCA GCA GAG GCT AGC CCC AAT CCT
 3844 3853 3862 3871 3880 3889

E I I T G T T D S P S N L F P S T S V P
 GAG ATC ATA ACT GGA ACC ACT GAC TCT CCC TCT AAT CTG TTT CCA TCC ACT TCT GTG CCA
 3904 3913 3922 3931 3940 3949

A L R V D K P Q N S K W K P S P W P E H
 GCA CTA AGG GTA GAT AAA CCA CAG AAT TCT AAA TGG AAG CCC TCT CCC TGG CCA GAA CAC
 3964 3973 3982 3991 4000 4009

K Y Q L K S Y S E T I E K G K R P A V S
 AAA TAT CAG CTC AAG TCA TAC TCC GAA ACC ATT GAG AAG GGC AAA AGG CCA GCA GTA AGC
 4024 4033 4042 4051 4060 4069

M S P H L S L P E A S T H A S H W N T Q
 ATG TCC CCC CAC CTC AGC CTT CCA GAG GCC AGC ACT CAT GCC TCA CAC TGG AAT ACA CAG
 4084 4093 4102 4111 4120 4129

FIG. 50

K H A E K S V F D K K P G Q N P T S K H
 AAG CAT GCA GAA AGT AGT GTT TTT GAT AAG AAA CCT GGT CAA AAC CCA ACT TCC AAA CAT
 4144 4153 4162 4171 4180 4189

 L P Y V S L P K T L L K K P R I I G G K
 CTG CCT TAC GTC TCT CTA CCT AAG ACT CTA TTG AAA AAG CCA AGA ATA ATT GGA GGA AAG
 4204 4213 4222 4231 4240 4249

 A A S F T V P A N S D V F L P C E A V G
 GCT GCA AGC TTT ACA GTT CCA GCT AAT TCA GAC GTT TTT CTT CCT TGT GAG GCT GTT GGA
 4264 4273 4282 4291 4300 4309

 D P L P I I H W T R V S S G X E I S Q G
 GAC CCA CTG CCC ATC ATC CAC TGG ACC AGA GTT TCA TCA GGA NTT' GAA ATA TCC CAA GGG
 4324 4333 4342 4351 4360 4369

 T Q K S R F H V L P N G T L S I Q R V S
 ACA CAG AAA AGC CGG TTC CAC GTG CTT CCC AAT GGC ACC TTG TCC ATC CAG AGG GTC AGT
 4384 4393 4402 4411 4420 4429

FIG. 5P

I	Q	D	R	G	Q	Y	L	C	S	A	F	N	P	L	G	V	D	H	F
ATT	CAG	GAC	CGT	GGA	CAG	TAC	CTG	TGC	TCT	GCA	TTT	AAT	CCA	CTG	GGC	GTA	GAC	CPT	TTT
					4453			4462			4471			4480			4489		
H	V	S	L	S	V	V	F	Y	P	A	R	I	L	D	R	H	V	K	E
CAT	GTC	TCT	TTG	TCT	GTG	GTT	TTT	TAC	CCG	GCA	AGG	ATT	TTG	GAC	AGA	CAT	GTC	AAG	GAG
					4513			4522			4531			4540			4549		
I	T	V	H	F	G	S	T	V	E	L	K	C	R	V	E	G	M	P	R
ATC	ACA	GTT	CAC	TTT	GGA	AGT	ACT	GTG	GAA	CTA	AAG	TGC	AGA	GTG	GAG	GGT	ATG	CCG	AGG
					4573			4582			4591			4600			4609		
P	T	V	S	W	I	L	A	N	Q	T	V	V	S	E	T	A	K	G	S
CCT	ACG	GTT	TCC	TGG	ATA	CTT	GCA	AAC	CAA	ACG	GTG	GTC	TCA	GAA	ACG	GCC	AAG	GGA	AGC
					4633			4642			4651			4660			4669		
R	K	V	W	V	T	P	D	G	T	L	I	I	Y	N	L	S	L	Y	D
AGA	AAG	GTC	TGG	GTA	ACA	CCT	GAT	GGA	ACA	TTG	ATC	ATC	TAT	AAT	CTG	AGT	CTT	TAT	GAT
					4693			4702			4711			4720			4729		

FIG.5Q

R G F Y K C V A S N P S G Q D S L L V K
 CGT GGT TTT TAC AAG TGT GTG GCC AGC AAC CCA TCT GGC CAG GAT TCA CTG TTG GTT AAG
 4744 4753 4762 4771 4780 4789

 I Q V I T A P P V I I E Q K R Q A I V G
 ATA CAA GTC ATC ACA GCT CCC CCT GTC ATT ATA GAG CAA AAG AGG CCA GCC ATC GTT GGG
 4804 4813 4822 4831 4840 4849

 V L G G S L K L P C T A K G T P Q P S V
 GTT TTA GGT GGA AGT TTG AAA CTG CCC TGC ACT GCA AAA GGA ACT CCC CAG CCT AGT GTT
 4864 4873 4882 4891 4900 4909

 H W V L Y D G T E L K P L Q L L T H S R F
 CAC TGG GTC CTT TAT GAT GGG ACT GAA CTA AAA CCA TTG CAG TTG ACT CAT TCC AGA TTT
 4924 4933 4942 4951 4960 4969

 F L Y P N G T L Y I R S I A P S V R G T
 TTC TTG TAT CCA AAT GGA ACT CTG TAT ATA AGA AGC ATC GCT CCT TCA GTG AGG GGC ACT
 4984 4993 5002 5011 5020 5029

FIG. 5R

Y E C I A T S S S G S E R R V I L T V
 TAT GAG TGC ATT GCC ACC AGC TCC TCA GGC TCA GAG AGA AGG GTA GTG ATT CTT ACT GTG
 5044 5053 5062 5071 5080 5089

E E G E T I P R I E T A S Q K W T E V N
 GAA GAG GGA GAG ACA ATC CCC AGG ATA GAA ACT GCC TCT CAG AAA TGG ACT GAG GTG AAT
 5104 5113 5122 5131 5140 5149

L G E K L L L L N C S A T G D P K P R I I
 TTG GGT GAG AAA TTA CTA CTG AAC TGC TCA GCT ACT GGG GAT CCA AAG CCT AGA ATA ATC
 5164 5173 5182 5191 5200 5209

W R L P S K A V I D Q W H R M G S R I H
 TGG AGG CTG CCA TCC AAG GCT GTC ATC GAC CAG TGG CAC AGA ATG GGC AGC CGA ATC CAC
 5224 5233 5242 5251 5260 5269

V Y P N G S L V V G S V T E K D A G D Y
 GTC TAC CCA AAT GGA TCC TTG GTG GTT GGG TCA GTG ACG GAA AAA GAC GCT GGT GAC TAC
 5284 5293 5302 5311 5320 5329

FIG. 55

[illegible]

FIG. 57

R	Y	"I"	L	F	H	N	G	T	L	Y	F	N	N	V	G	M	A	E	E
AGG	TAC	ACC	CTT	TTC	CAC	AAT	GGA	ACC	TTG	TAT	TTC	AAC	AAC	GTT	GGG	ATG	GCA	GAG	GAA
		5584		5593			5602				5611			5620			5629		
G	D	Y	I	C	S	A	Q	N	T	L	G	K	D	E	M	K	V	H	L
GGA	GAT	TAT	ATC	TGC	TCT	GCC	CAG	AAC	ACC	TTA	GGG	AAA	GAT	GAG	ATG	AAA	GTC	CAC	CTA
		5644		5653			5662				5671			5680			5689		
T	V	L	T	A	I	P	R	I	R	Q	S	Y	K	T	T	M	R	L	R
ACA	GTT	CTA	ACA	GCC	ATC	CCA	CGG	ATA	AGG	CAA	AGC	TAC	AAG	ACC	ACC	ATG	AGG	CTC	AGG
		5704		5713			5722				5731			5740			5749		
A	G	E	T	A	V	L	D	C	E	V	T	G	E	P	K	P	N	V	F
GCT	GGA	GAA	ACA	GCT	GTC	CTT	GAC	TGC	GAG	GTC	ACT	GGG	GAA	CCG	AAG	CCC	AAT	GTA	TTT
		5764		5773			5782				5791			5800			5809		
W	L	L	P	S	N	N	V	I	S	F	S	N	D	R	F	T	F	H	A
TGG	TTG	CIG	CCT	TCC	AAC	AAT	GTC	ATT	TCA	TTC	TCC	AAT	GAC	AGG	TTC	ACA	TTT	CAT	GCC
		5824		5833			5842				5851			5860			5869		
N	R	T	L	S	I	H	K	V	K	P	L	D	S	G	D	Y	V	C	V
AAT	AGA	ACT	TTG	TCC	ATC	CAT	AAA	GTG	AAA	CCA	CTT	GAC	TCT	GGG	GAC	TAT	GTC	TGC	GTA
		5884		5893			5902				5911			5920			5929		

FIG. 5U

A	Q	N	P	S	G	D	D	T	K	T	Y	K	L	D	I	V	S	K	P
GCT	CAG	AAT	CCT	AGT	GGG	GAT	GAC	ACT	AAG	ACA	TAC	AAA	CTG	GAC	ATT	GTC	TCT	AAA	CCT
					5953			5962			5971			5980			5989		
					5944														
P	L	I	N	G	L	Y	A	N	K	T	V	I	K	A	T	A	I	R	H
CCA	TTA	ATC	AAT	GGC	CTG	TAT	GCA	AAC	AAG	ACT	GTT	ATT	AAA	GCC	ACA	GCC	ATT	CGG	CAC
					6013			6022			6031			6040			6049		
					6004														
S	K	K	Y	F	D	C	R	A	D	G	I	P	S	S	Q	V	T	W	I
TCC	AAA	AAA	TAC	TTT	GAC	TGC	AGA	GCA	GAT	GGG	ATC	CCA	TCT	TCC	CAG	GTC	ACG	TGG	ATT
					6073			6082			6091			6100			6109		
					6064														
M	P	G	N	I	F	L	P	A	P	Y	F	G	S	R	V	T	V	H	P
ATG	CCA	GGC	AAT	ATT	TTC	CTC	CCA	GCT	CCA	TAC	TTT	GGA	AGC	AGA	GTC	ACG	GTC	CAT	CCA
					6133			6142			6151			6160			6169		
					6124														
N	G	T	L	E	M	R	N	I	R	L	S	D	S	A	D	F	T	C	V
AAT	GGA	ACC	TTG	GAG	ATG	AGG	AAC	ATC	CGG	CTT	TCT	GAC	TCT	GCG	GAC	TTC	ACC	TGT	GTG
					6184			6202			6211			6220			6229		
					6193														

FIG. 5V

V R S E G G GAG GGA GGA GAG AGT GTG TTG GTA GTG CAG TTA GAA GTC CTA GAA ATG CTG
 GTT CGG AGC GAG GAG GGA GGA GGA GAG AGT GTG TTG GTA GTG CAG TTA GAA GTC CTA GAA ATG CTG
 6244 6253 6262 6271 6280 6289

R R P T F R R N P F N E K V I A Q A G K P
 AGA AGA CCA ACA TTC AGA AAC CCA CCA TTC AAC GAA AAA GTC ATC GCC CAA GCT GGC AAG CCC
 6304 6313 6322 6331 6340 6349

V A L N C S V D G N P P P P E I T W I L P
 GTA GCA CTG AAC TGC TCT GTG GAT GGG AAC CCC CCA CCT GAA ATT ACC TGG ATC TTA CCT
 6364 6373 6382 6391 6400 6409

D G T Q F A N R P H N S P Y L M A G N G
 GAC GGC ACA CAG TTT GCT AAC AGA CCA CAC AAT TCC CCG TAT CTG ATG GCA GGC AAT GGC
 6424 6433 6442 6451 6460 6469

S L I L Y K A T R N K S G K Y R C A A R
 TCT CTC ATC CTT TAC AAA GCA ACT CGG AAC AAG TCA GGG AAG TAT CGC TGT GCA GCC AGG
 6484 6493 6502 6511 6520 6529

FIG. 5W

N K V G Y I E K L I L L L E I G Q K P V I
 AAT AAG GTT GGC TAC ATC GAG AAA CTC ATC CTG TTA GAG ATT GGG CAG AAG CCA GTC ATT
 6544 6553 6562 6571 6580 6589

 L T Y E P G M V K S V S G E P L S L H C
 CTG ACA TAC GAA CCA GGG ATG GTG AAG AGC AGT GTC AGT GGG GAA CCG TTA TCA CTG CAT TGT
 6604 6613 6622 6631 6640 6649

 V S D G I P K P N V K W T T P G G H V I
 GTG TCT GAT GGG ATC CCC AAG CCA AAT GTC AAG TGG ACT·ACA CCG GGT GGC CAT GTA ATC
 6664 6673 6682 6691 6700 6709

 D R P Q V D G K Y I L H E N G T L V I K
 GAC AGG CCT CAA GTG GAT GGA AAA TAC ATA CTG CAT GAA AAT GGC ACG CTG GTC ATC AAA
 6724 6733 6742 6751 6760 6769

 A T T A H D Q G G A AAT TAT ATC TGT AGG GCT CAA AAC AGT GTT GGC CAG
 GCA ACA ACA GCT CAC GAC CAA GGA AAT TAT ATC TGT AGG GCT CAA AAC AGT GTT GGC CAG
 6784 6793 6802 6811 6820 6829

FIG. 5X

A	V	I	S	V	S	V	M	V	V	A	Y	P	P	R	I	I	N	Y	L	
GCA	GTT	ATT	AGC	GTG	TCA	GTG	ATG	GTT	GTG	GCC	TAC	CCT	CCC	CGA	ATC	ATA	AAC	TAC	CTA	
	6844				6853			6862			6871			6880					6889	
P	R	N	M	L	R	R	T	G	E	A	M	Q	L	H	C	V	A	L	G	
CCC	AGG	AAC	ATG	CTC	AGG	AGG	ACA	GGG	GAA	GCC	ATG	CAG	CTC	CAC	TGT	GTG	GCC	TTG	GGA	
	6904				6913			6922			6931			6940					6949	
I	P	K	P	K	V	T	W	E	T	P	R	H	S	L	L	S	K	A	T	
ATC	CCC	AAG	CCA	AAA	GTC	ACC	TGG	GAG	ACG	CCA	AGA	CAC	TCC	CTG	CTC	TCA	AAA	GCA	ACA	
	6964				6973			6982			6991			7000					7009	
A	R	K	P	H	F	S	E	M	L	H	P	Q	G	T	L	V	I	Q	N	
GCA	AGA	AAA	CCC	CAT	AGA	AGT	GAG	ATG	CTT	CAC	CCA	CAA	GGT	ACG	CTG	GTC	ATT	CAG	AAT	
	7024				7033			7042			7051			7060					7069	
L	Q	T	S	D	S	G	V	Y	K	C	R	A	Q	N	L	L	G	T	D	
CTC	CAA	ACC	TCG	GAT	TCC	GGA	GTC	TAT	AAG	TGC	AGA	GCT	CAG	AAC	CTA	CTT	GGG	ACT	GAT	
	7084				7093			7102			7111			7120					7129	

FIG. 5Y

[illegible]

72/183

FIG. 5Z

TAC GAA ATA ACA AAC GGC TAA TGC ACC TGA ATT CTC AGT AAA AAG ACC TTT CTC TCG CTA
 7444 7453 7462 7471 7480 7489

ACA GTT GCC AGC TGC CTC GTG TCT GTT TCC TAC CAA TGT CAC AAA CAT CGC ACA CAG GGT
 7504 7513 7522 7531 7540 7549

GAA TGG AGT CAA CGG GAA AGA TTA AGT TTG CGG TCT GTG TAA ATC TCA ATG TAC AAA TAT
 7564 7573 7582 7591 7600 7609

TCT GTC NCT GGT TTA TAA ACA [?] TTT TGA TAA AAC CGA AAA AAA AAA AAA AAA AAA
 7624 7633 7642 7651 7660 7669

AAA AA

FIG. 6A

608: CLUSTAL X (1.64b) Multiple Sequence Alignment

gi 1017427 emb CAA62189	PDQEMPVYPPAIITPLQDVTVTSEGPQPARFCQRCRVSGTDLKVSWSKDKKIK
gi 3328186	-MGRSPSWIYGVLGLLLATT-----CSSVNDKND---PTGKSS
608	-----
gi 1017427 emb CAA62189	PSRFFRMT--QFEDTYQLEIAEAYPEDEGTYTFVANNVAVGQVSSTANLSL
gi 3328186	LAFVFDITGSMFDDLVQVREGAAK-----IFKTVMAQREKLIYNYIMVPF
608	-----
gi 1017427 emb CAA62189	EAPES--ILHERIEQEIEMEMKAAPVIKRKIEPLEVALGHLAKFTCEIQS
gi 3328186	HDPYIGEIINTTDSYFMRQLSKVYVHGGDCPEKTLTGILKALQISLPS
608	-----
gi 1017427 emb CAA62189	APNVRQWFKAGREIYESDKCSIRSSKYISSLEILRTQVVDCGEYTCAS
gi 3328186	SFIYVFTDARSKDYHLEDEVLTIQEK-QSS--VVFVMTGDCGNRTHPGF
608	-----
gi 1017427 emb CAA62189	NEYGSVSTATLTVTEAYPPTEFLSRPKSLTTFVCGKAAKFICTVTGTPVIE
gi 3328186	RTYEKIAAAS-----FGQVFHLEKSDVSTVL---EYVRHAVKQKKVH
608	-----

FIG. 6B

gi 1017427 emb CAA62189 gi 3328186 608	TIWQKGAALSPSPNWRI SDAENKHI LELSNLT IQDRGVYSCASNKFGA LMYERERGGTVSRNIPV-----DKHLSLTISLSGDK-----DDSDNLDI -----
gi 1017427 emb CAA62189 gi 3328186 608	DICQAEIIIDKPHFIKELEPVQSAINKKVHLEQCQVDEDRKVTVTWSKDG VLRDPEGR TVDKRLYSKEGGTIDLKNVKLIRLKDPSPGVWTVNTN-SRLK -----
gi 1017427 emb CAA62189 gi 3328186 608	QKLPPGKDYKICFEDKIATLEIPLAKLKDSGT YVCTASNEAGSSSCSATV HTIRVFGHGA VDFKYGFASRPLDRIELARPRPVLNQDT-----YLLINM -----
gi 1017427 emb CAA62189 gi 3328186 608	TVREPPSFVKKVDPSYIMLPGESARLHCKLKGSPVIQVTFKNNKELSES TGLIPPGTVGEID--LVDYHG-----HSLYKAVASPHRT-NPNMYFAGPF -----
gi 1017427 emb CAA62189 gi 3328186 608	NTVRMYFVNSEAILDITDVKVEDSGSYSCA VNDVGS DSCSTEI VIKPEPP VPPKGLFF---VRVQGYD---EDNYEFMRIAFTAIGS-----VIVGGPR -----

FIG. 6C

gi 1017427 emb CAA62189 gi 3328186 608	SFIKTLPADIVRGTNALQCEVSGTGPFEISWFKDKKQIRSSKKYRLFS AFMSPIHQEFVGRDLN--LSCTVESASAYTIYWVKTGEDIIGGPLFYHNT -----
gi 1017427 emb CAA62189 gi 3328186 608	OKSLVCLCIFSFNSADVGEYECVVANEVGKCGCMATHLLKEPPTFVKKVD DTSVWTIPELSLK--DAGEYECRVISNNGNYSVKTRVETRESPPEIFGVR -----
gi 1017427 emb CAA62189 gi 3328186 608	PLIALGGQTVTLQAAVRGSEPISVTWMKGQEVIREDGKIKMSFSNGVAVL NVSVP LGEAAFLHCSTRSAGEVEIRWTRYGATVFNPNTERNPTNG--TL -----
gi 1017427 emb CAA62189 gi 3328186 608	IIPDVQISFGGKYTCLAENEAGSQTSV GELIVKEPAKIERAELIQVTAG KIHVTRADAGVYECMARNAGGMSTRKMRLDIMEPPSVKVTPQDVYFNMR -----
gi 1017427 emb CAA62189 gi 3328186 608	DPATLEYTVAGTPELKPWKYKDG RPLVASKKYRISFKNNVAQLKFYSAEL EGVNLSCAMGDPKPEVHWYFKGRHLLNDYKYQVGQDS--KFLYIRDATH -----

FIG. 6D

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

HDGQYTFEISNEVGSSCETFTVLDRDIAPFFTKPLRNVDVVNGTCR
HDEGTYECRAMSQAG---QARDTTDIMLATPPKVEIIQNKMVGRGDRV

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

-LDCKIAGSLPMR-VSWFKDGKEIAASDRYIAFVEGTASLEIIRVDMND
SFECKTIRGKPHPKIRWFKNKGKDLIKPDDY-IKINEG--QLHIMGAKDED

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

AGNFTCRATNSVGSKDSSGALIVQEPSPFVTKPGSKDVLPGSAVCLKSTF
AGAYSCVGENMAGKDVQVANLSVGRVPTIIESPHTVRVNIERQVTLQCLA

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

FVKEPAAFLKRLSDHSVEPGKSIILESTYTGTLPISVTWKKGFNITTS
VIIPPVIDGERREAVAVIEGFSSELCDSNSTG-VDVEWQKGLTINQDT
LPLSARRIDPQRWAAALLEKAKKNSVPPKKQENTTVKPVPLAVPLVELTDEE
. : *

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

KCN---IVTTEKTCILEILNSTKRDAGQYSCIEIENEAGRDCGALVSTLE
LRGDSFIQIPSSGKKMSFLSARKSDSGRYTCIVRNPAAGEARKLFDEAVND
KDAS---GMIPPDDEEFMVLKTKASGVPGRSPTADSGPVNHGFMITSIASGTE
. : * :

FIG. 6E

gi 1017427 emb CAA62189	PPYFVTELEP--LEAAVG-DSVSLQCQVAGTPEITVSWYKGDTKLRPTPE
gi 3328186	PPSIDELSSANIQTIVPYYPVEINC VVSGSPHPKVYWLFD DDKPLEPD-S
608	VSTVNPQTLQSEHLPDFKLF SVTNGTAVTKSMNP SIA SKIEDTTNQNP II
gi 1017427 emb CAA62189	YRTYFTNNVATLVFNKVNINDSGEYTC KAEN SIGTASSKTVFRIQERQLP
gi 3328186	AAVELTNNGETLKI VRSQVEHAGT YTCEAQN NVGKARKDFLVRVT---AP
608	IFPSVAEIRDSAQAGRASSQSAHPVTGGNMATYGH TINTYSSFTSK-----
gi 1017427 emb CAA62189	PSFARQLKDI EOTVGLPVTLTCRLNGSAPIQVCWYRDGVLLRDHENLQTS
gi 3328186	PHFEKEREEVVARVGD TMLLTCAESSVPLSSVYWHAHDESVQNGVITSK
608	-----ASTVLQPINPTESYGPQIPITGVSRPSSD ISSHTTADPS
gi 1017427 emb CAA62189	FVDNVATLKILQTDLSHSGQYSCSASNPLGTASSSARLTAREPKKSPFED
gi 3328186	YAANEKTLNVNTNIQLDDEGFYYCTAVNEAGITKFFKLIV IET---PYFL
608	FSSHIPSGSHTTASSL FHI PRNNNTGNFPLSRHLGRERTIWSRG-----RV-
gi 1017427 emb CAA62189	IKPVSIDVIAGESADFECHVTGAQPMRI TWSKDNKEIRPGGNYTITCVGN
gi 3328186	DQOKLYPIILGKRLTLD CSATGTPPTILFMKDGK--RINESDEVDIIGS
608	KNPHRTPVLRHRRHRTVRPAIKGPANKVNSQVPATEYPGMCHTCPSAEGL

FIG. 6F

gi 1017427 emb CAA62189	TPHLRILKVGKDSGQYTCQATNDVGKDMCSAQLSVKEPPKFKVKKLEASK
gi 3328186	T--LVIDNPQKEVEGRYTCIAENKAGRSEKDMVVEVLLPPKLSKEWINVE
608	T--VATAALSVPSSSHSAIPKTNVGVIAEESTTVVKKPELLLFKDKQNVD
	* : : : : * * * * *
gi 1017427 emb CAA62189	VAKGESIQLECKISGSPEIKVSWERNDSSELHESWKYNMFSINSVALLTI
gi 3328186	VQ-AGDPLTLECPIEDTSGVHI TWSRQFGKDGQLDMRAQSSSDKSKLY-I
608	IE-----IITTTTKYSGGESNHV IPTAS-----MTSAPTSVSLG--
	: : : : : *
gi 1017427 emb CAA62189	NEASAEDSGDYICEAHNGVGDA SCSTALTVKAPP-VFTQKPSVPGALKGS
gi 3328186	MQATPEDADSYSCIAVNDAGGA EAVFQVTVNTPPKIFGDSEFSTTEIVADT
608	--KSPVDNS-----GHLSMPGT IQTGKDSVETTP---LPSPLSTPSIPTS
	: * . . . : : * : : * : : *
gi 1017427 emb CAA62189	DVILQCEISGTPPFVWVVKDRKQVRNSKKFKITSKHFDTNLHILNLEAS
gi 3328186	TLEIPCRTEGIPPEISWFLDGK-----PIL EMP--
608	TKFSKRKTPLHQIFVNNQKKEGM-----LKNP--
	: : *
gi 1017427 emb CAA62189	DVGEYHCKATNEVGSDTCSCSVKFKKEPPRFVKKLSDTSTLIGDAVELRAI
gi 3328186	---G-----VTYK---Q-----GD-----
608	-----YQFG-----
	:

FIG. 6G

gi 1017427 emb CAA62189 gi 3328186 608	VEGFQPISVVWLKDRGEVIRESENTRISFIDNIATLQLGSPEASNSGKYI -----LSLR-----IDNIKPNEG-----RYT -----LQKNPAAKLP-----KIA ::: . . .
gi 1017427 emb CAA62189 gi 3328186 608	COIKNDAGMRECSAVLTVLEPARIIEKPEPMVTVTGNPEALECVVTGTPE CVAENKAGRAEQDTYVEISEPPRVVMASEVMRVVEGRQTTIRCEVFGNPE PLLP TGQSSPSDSTLLTSPPP---ALSTTMAATQNKGTENVVGARSLSA * :
gi 1017427 emb CAA62189 gi 3328186 608	LSAKWFKDGRELSADSKHHITFINKVASILKIPCAEMSDKGLYSFEVKNSV PVVNWLDGGEPTYSD---LLOFSTKLSYLHLRETTLADGGTYTCIATNKA GKKQPFNTNSSPVLPS-----TISKRSNTLNFLSTETPTVTSPTATASVIM : : : : : : : :
gi 1017427 emb CAA62189 gi 3328186 608	GKSNCTVSVHVSDRIVPPSFIRKLKDVNAI LGASVVLECRVSGSAPISVG GESQTTTDVEV---LVPPRIEDEERVLOGKEGNTYMHVCQVGTGRPVYVT SETQTRSKEA-----KDQIKGPRKNRNNANTTPROVSG-----ISA ::: * : : : * : *
gi 1017427 emb CAA62189 gi 3328186 608	WFQDNEIVSGPKCQSSFSENVCTLNLSLLEPSDTGIYTCVAANVAGSDE WKRNGKEIEQ-----FNP---VLHIRNATRADEGKYSCIASNEAGTAV YSALTTTADTP-----LAFSHSPRQDDG-----G---NVSAVAY : * : * * * * : . .

```

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

gi | 1017427 | emb | CAA62189 |
gi | 3328186
608

```

FIG. 6I

gi|1017427|emb|CAA62189|
gi|3328186
608

gi|1017427|emb|CAA62189|
gi|3328186
608

gi|1017427|emb|CAA62189|
gi|3328186
608

gi|1017427|emb|CAA62189|
gi|3328186
608

gi|1017427|emb|CAA62189|
gi|3328186
608

KNNVASLVINKVDHSDVGEYSC:KADNSVGAVASSAVLVIKARKLPPFFAR
DG--SHITINKAKLSDGGKYICRASNEAG---TSDIDLILKILVPPKIDK
KH-----AEKSVFDKKPG--QNPTSKHLP-----YVSLPKTLLKKPRIIG
. * : : : : : * :

K--LKDVHETLGFPPVAFECRINGSPELQVSWYKDGVLKDDANLQTSFVH
SNIIIGNPLAIVARTIYLECPISGIPQPDVIWTKNGMDIN-MTDSRVILAQ
G-KAASFTVPANSDVFLPCEAVGDPLPIIHWTVRSSGXEISQGTQKSRFH
. * : : : : : * :

NVA--TLQILQTDQSHIGQYNCSASNPLGTASSAKLILSEHEVPPFFDL
NNE--TFGIENVQVTDQGRYTCTATNRGGKASHDFSLDVLS---PPEFDI
VLPNGTILSIQRVSIQDRGQYILCSAFNPLGVDFHVSLSVVFYP-ARILDR
* : * * : * * * * . * : * :

KPVSVDLA-LGESGTFKCHVTGTAPIK-----ITWAKDNREIR---PGGN
HGTQPTIKREGDTITLTCPIKLAEDIADQVMDVSWTKDSRALD---GDLT
HVKEITVH-FGSTVELKCRVEGMPRPT-----VSWILANQTVVSETAKGS
. * : * : * : * : * : * : * : * :

YKMTLLVENTATLTVLKVGKGDAGQYTCYASNIAGKSCSAQLGVQEPFR
DNVDISDDGRKLTISQASLENAGLYTCTIALNRAGEASLEFKVEILSPPI
RKVWVTPDG-TLIIYNLSLYDRGFYKCVASNPSGQDSSLVVKIQVITAPPV
: : : : * : : : : * : * : * : * : * : * : * :

FIG. 6K

gi 1017427 emb CAA62189	--WISYENIATLQFSRVEPANAGKYTCQIKNDAGMQECFATLSVLEPAT
gi 3328186	--GITVASDSTFIQINNVSLSDKGVYTCYAENVAGSDNLMYNVDVVQ-AP
608	RTKRYTLFHNGTLYFNNVGMAGEEGDYICSAQNTLGKDEMKVHLTVLTAIP
	. : :...* : : * * * * : * : * : *
gi 1017427 emb CAA62189	IVEKPES--IKVTTGDTCTLECTVAGTPELSTKWFKDGKELTSDNKYKIS
gi 3328186	VISNGGT--KQVIEGELAVIECLVEGYAPQVSWLRNGNRVETG-VQGV
608	RIRQSYKTTMRLRAGETAVIDCEVTGEPKPNVFWLLPSNNVISE-SNDRF
	: : . : : * : : : * * * * : * : : : *
gi 1017427 emb CAA62189	FFNKVSGLKIIINVAPSDSGVYSFEVQNPVGKDSCTASLQVSDRTVPPSFT
gi 3328186	YVTDGRMLTIIIEARSLDSGIYLCSATNEAG--SAQQAYTLEVLVSPKIIT
608	TFHANRTLSIHKVKPLDSGDYVCVAQNPSG--DDTKTYKLDIVSKPPLIN
	. : : . *** * . * * . : : . : *
gi 1017427 emb CAA62189	RKLK-----ETNGLSGSSVWMECKVYGSPPIISVSWFHEGNEISSGRKYQI
gi 3328186	STPG-----VLTPSSGSKFSLPCAVRGYPDPPIISWTLNGNDIKDGENGHT
608	GLYANKTVIKATAIRHSKKYFDCRADGIPSSQVTWIMPGNIFLPAPYFGS
	*. : : * * * * : * : * : *
gi 1017427 emb CAA62189	TLTDNTC-ALTVNMLEESDSGDYTCIATNMAGSDECSAPL-----TVREPP
gi 3328186	IGADG----TLHIEKAEER-HLIYECTAKNDAGADTLEFPVQ----TIVAPK
608	RVTVHPNGTLEMRNIRLSDSADFTCVVRSEGESVLVQLEVLEMLRRPT
	: * : : . : * . . . * . : : *

FIG. 6L

gi 1017427 emb CAA62189	JFVQKPDMDVLTGNTVTFTSIVKGTFFFSVSWEK-GSSELVPGDRCNVS
gi 3328186	ISTSGNRYINGSEGTETVIKCEIE-SESSEFSWSK-NGVPLLP SN--NLI
608	FRNPFNEKVIAQAAGKPVALNCSDGNPPPEITWILPDGTQEFANRPHNSPY
	* : * : * : * :
gi 1017427 emb CAA62189	LEDVAELELFVDVTSQSGETCIVSNEAGKASCTTHLYIKAPAKFVKRL
gi 3328186	FSEDYKLIKILSTRLSHQGEYSCTAANKAGNATQKTNLNVGVAPKIMERP
608	LMAGNGSLILYKATRNGSGKYRCAARNKVGYIEKLI LLEIGQKPVILTYE
	: : * : * : * :
gi 1017427 emb CAA62189	NDYSIEK-GKPLILEGFTGTPPISVTWK-KNGINVTSPQRCNITTEKS
gi 3328186	RTQVVHK-GDQVTLWCEASGVQPQAI TWY-KDNELLTNTGVDETATTKK
608	PGMVKSVSSEPLSLHCVSDGIPKPNVKWTPGHHVIDRPQVDGKYILHEN
	* : * : * : * :
gi 1017427 emb CAA62189	PILEIPSTVEDAGQYNCYIENASGKSDSCSAQILILEPPYFVKQLEPVKV
gi 3328186	SVIFSSISPSQ-AGVYTCKAENWVASTEEDIDLIVMIPPEVVPERMNVST
608	GTLVIKATTAHDQGNYYICRAQNSVGQAVISVSVMVVAYPPRI INYLPRNM
	: : * : * : * :
gi 1017427 emb CAA62189	S VGDSASLQCQLAGTPEIGVSWYKG-DTKLRPTTTYKMHFRNNV---A
gi 3328186	N--PRQTVFLSCNATGIPEPVI SWMR--DSNIAIQNNEKYQILG-----T
608	LRRTGEAMQLHCVLALGIPKPKVTTWETPRHSLLSKATARKPHRSEMLHPQG
	:: * * * * * : * * * * * : * * * * * : * * * * * :

FIG. 6M

gi | 1017427 | emb | CAA62189 |
 gi | 3328186
 608

TLVFNQVDINDSGEYICKAENSVGEVSASTFLTVQEQKLPPSFRQLRDV
 TI, A I R N V L P D D D G F Y H C I A K S D A G Q I A T R K L I V N K P S D R P -----
 T L V I Q N L Q T S D S G V Y K C R A Q N L L G T D Y A T T Y I Q V L -----
 * * * * * : * * * * * : * * * * * : *

gi | 1017427 | emb | CAA62189 |
 gi | 3328186
 608

QETVGLPVVFDCAISGSEPI SVSWYKDGKPLKDSPNVQTSFLDNTATLNI
 -----A P I W V E C D E K G - K P K K T E Y M I D R -----G D T P -----D D N P Q L L P W -----

gi | 1017427 | emb | CAA62189 |
 gi | 3328186
 608

FKT-DRSLAG--QYSCATNPIGSASSSARLILTEGKNPPFFDIRLAPVD
 KDVEDSSLNGSLAYRCMPG-PR-----SSRTVLLH--AAPQFIVKPKNTT

gi | 1017427 | emb | CAA62189 |
 gi | 3328186
 608

AVVGESADFECHVTGTQPIKVSWAKDSREIRSGGKYQISYLENSAHLTVL
 AAIGAIVELRCSAAGPPHPTITWAKDGKLI EDS-KFEIAY-----SHLKVT

gi | 1017427 | emb | CAA62189 |
 gi | 3328186
 608

KVDKGDSGQYTCYAVNEVGKDSCTAQLNIKERLIP---PSFTKRLSETVE
 INSTSDSGEYTCMAQNSVGSTVSAFINVDNNILPTPKPSSNQKNVAVIT

FIG. 6N

gi 1017427 emb CAA62189 gi 3328186 608	ETEGNSFKLEGRVAGSQPITVAWYKNNIEIQPTSNCEITFKNN--TLVLQ CYERNQAYSRG-----LTWEYNGVP-MPKNLAGIHFMNNGSLVILD -----
gi 1017427 emb CAA62189 gi 3328186 608	VRKAGMNDAGLYTCKVSNDAGSALCTSSIVIKEPKPPVFDQHLTPVTVS TSSLKEGDLELYTCKVRNR-----RHSIPHLTS -----
gi 1017427 emb CAA62189 gi 3328186 608	EGEYVQLSCHVQSEPIRIQWLKAGREIKPSDRCSFSFASGTAVLELRDV AFEGVP-----EVTID-----KVEV -----
gi 1017427 emb CAA62189 gi 3328186 608	AKADSGDYVCKASNVAGSDTTKSKVTIKDKPAVAPATKKAADVGRLEFFVS NNGDSVVLDC-----EVT-----S -----
gi 1017427 emb CAA62189 gi 3328186 608	EPQSI RVVEKTTATFI AKVGGDPI PNVKWTGKWRQLNQGGRVFIHQKGD DP--LTT-----HVVTKNQDKMLDDDAIYVLP----- -----

FIG. 60

gi 1017427 emb CAA62189 gi 3328186 608	EAKLEIRDTTKTDSGLYRCVAFNEHGEIEESNVNLQVDERKKQEKIEGDLR NNSLVLLNVEKYDEGVYKCVASNSIGKAFDDTQLNVY-----EGDFL -----
gi 1017427 emb CAA62189 gi 3328186 608	AMLKKTPIILKKGAGEEEIDIMELLKNVDPKEYEKYARMYGITDFRGLLQ PLTG-----FE-GSG-----IN-IDD-----SSNAGGSSRR----- -----
gi 1017427 emb CAA62189 gi 3328186 608	RVEEEHVRVEKVRVIEVFEAEVEVEFEKPKAPKGPSEKIIPPKKPPT ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	KVPRKEPPAKVPEVPKKIVVEEKVRVPEEPRVPTTKVPEVLPPEVPE ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	KKVPVPPAKKPEAPPKVPEAPKEVVPEKKVPVPPPKKPEVPPTKVPEVP ----- -----

FIG. 6P

gi 1017427 emb CAA62189 gi 3328186 608	KAAVPEKKVPEAIPPKPESPPPEVFEEPEESPSAPPKKPEVPPVRVPEVP ----- ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	KEVVPEKKVPAAPPKPEVTIPVKVPEAPKEVWPEKKVPVPPPKPEVPPPT ----- ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	KVPEVPKVAVPEKKVPEAIPPKPESPPPEVFEEPEEVALEPPAEVVEEP ----- ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	EPAAPQVTVPPKNPVPPEKKAPAVVAKKPELPFVKVPEVPKEVWPEKKVP ----- ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	LVVPEKKPEAPPKVPPEVPKEVWPEKKVAVPKKPEVPPAKVPEVPKKPVLE ----- ----- -----

FIG. 6Q

gi 1017427 emb CAA62189 gi 3328186 608	EKPAVPVPERAESPPPEVYEEPEEEIAPEEEIAPEEEKTPVVAEEEEPEVP ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	PPAVPEEPKKIIPKPKVPVIKKPEAPPPKEPEPEKVIKPKLKRPPPPPP ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	PAPPKEDVKEKIFQLKAIKKKVPENPQVPEKVELTPLKVPGGGKKVRKL ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	LPERKPEPKKEEVLKSVLRKRPEEEEPKVEPKLEKVKKPAVPEPPPPKP ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	VEEVEVPTVTKRERKIPEPTKVPEIKPAIPLPAPEPKPKEAEVKTIKPP ----- -----

FIG. 6R

gi 1017427 emb CAA62189 gi 3328186 608	PVEPEPTPIAAPVTVPVVGKKAEEAKAPKEEAAKPKGPIKGVPKKTPSPIE ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	AERRKLRPGSGGEKPPDEAPFTYQLKAVPLKFVKEIKDIIILTESEFVGSS ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	AI FECLVSPSTAITTWMKDGSNIRESPKHRFIADGKDRKLHIIDVQLSDA ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	GEYTCVLRGNKEKTSTAKLVVEELPVRFVKTLEEEVTVVKGQPLYLSCE ----- -----
gi 1017427 emb CAA62189 gi 3328186 608	LNKERDVVWRKDGKIVVEKPGRIVPGVIGLMRALTINDAD ----- -----

405 Human

PROBE: Human 405 EST Probe.

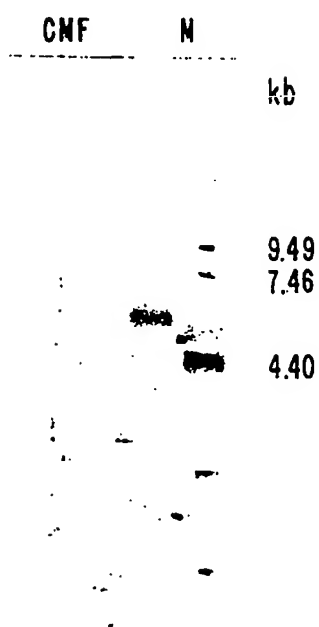
Target: total RNA: Human K562



FIG. 7

92/183

405 Human

Probe: Human 405 EST ProbeTarget: Rat Cmf mRNA**FIG. 8**

Length of 405-without up: 3722 bp; Listed from: 3 to: 3722;
Translated from: 48 to: 3176 (Entire region);
Genetic Code used: Universal;

[illegible]

FIG. 9B

W	F	L	P	P	C	M	A	H	C	I	R	L	A	L	I	R	F	H
TGG	TTC	CTG	CCT	CCG	GTC	TGC	ATG	GCC	CAC	TGC	ATC	CGC	CTG	GGC	CTC	ATC	CGC	TTC
		245			254			263		272			281					
V	K	V	A	Q	S	I	E	D	H	H	Q	E	V	I	G	F	C	E
GTC	AAG	GTT	GCA	CAG	AGC	ATT	GAG	GAT	CAT	CAT	CAA	GAA	GTG	ATT	GGC	TTC	TGC	AGA
		305			314			323			332			341			350	
N	G	F	H	G	L	V	A	Y	D	S	D	Y	A	L	C	N	I	P
AAT	GGT	TTC	CAT	GGC	TTG	GTG	GCG	TAT	GAC	TCT	GAT	TAT	GCA	CTG	TGC	AAC	ATC	CCC
		365			374			383			392			401			410	
Y	F	S	A	H	A	L	K	L	S	R	N	G	K	S	L	T	T	S
TAC	TTC	AGT	GCC	CAT	GCC	CTG	AAA	CTG	AGC	CGC	AAT	GGG	AAG	AGT	CTC	ACC	ACA	AGC
		425			434			443			452			461			470	
Y	L	M	H	E	V	A	K	Q	L	D	L	N	P	N	R	F	P	I
TAT	CTG	ATG	CAC	GAG	GTT	GCC	AAG	CAA	CTG	GAC	CTG	AAC	CCA	AAC	CGT	TTT	CCT	ATT
		485			494			503			512			521			530	

FIG.9C

A	A	L	L	G	N	H	I	L	P	D	E	D	L	A	S	F	H	W	S
GCT	GCT	CTG	TTA	GGA	AAT	CAC	ATA	CTA	CCT	GAC	GAG	GAT	CTG	GCT	TCC	TTT	CAT	TGG	AGT
		545		554				563			572			581			590		
L	L	G	P	E	H	P	L	A	S	L	K	V	R	A	H	Q	L	V	L
TTA	CTT	GGT	CCA	GAG	CAT	CCG	CTC	GCC	TCA	CTG	AAG	GTC	CGA	GCT	CAC	CAG	CTG	GTC	TTC
		605		614				623			632			641			650		
P	P	C	D	V	V	I	K	A	V	A	D	Y	V	R	N	I	H	D	T
CCA	CCA	TGT	GAT	GTA	GTG	ATC	AAA	GCT	GTA	GCT	GAT	TAT	GTC	CGC	AAC	ATC	CAT	GAC	ACG
		665		674				683			692			701			710		
S	D	L	D	A	I	A	K	D	V	F	Q	H	S	Q	S	R	T	D	D
TCT	GAC	CTG	GAT	GCC	ATA	GCT	AAA	GAT	GTT	TTC	CAG	CAT	TCA	CAG	TCT	AGA	ACA	GAT	GAC
		725		734				743			752			761			770		
K	V	I	R	F	K	R	A	V	G	Y	Y	S	A	T	S	K	P	M	P
AAA	GTC	ATT	CGA	TTT	AAG	AGA	GCA	GTT	GGA	TAT	TAC	TCA	GCA	ACT	AGC	AAG	CCT	ATG	CCA
		785		794				803			812			821			830		

FIG. 9D

F	H	P	P	P	H	P	P	L	P	R	P	N	P	F	G	M	P	G	M	V	P
TTT	CAC	CCA	CCA	CCA	CAT	TAC	TTA	CCC	CGA	CCA	AAT	CCA	TTT	GGA	ATG	CCT	GGG	ATG	GTG	CCA	
		845			854				863			872							881	890	
P	Y	V	P	P	P	Q	M	L	N	I	P	Q	Q	T	S	L	Q	A	K	P	A
CCA	TAT	GTT	CCC	CCC	CCT	CAG	ATG	CTC	AAC	ATT	CCA	CAG	CAG	ACT	TCT	CTG	CAA	GCA	AAG	CCC	GCG
		905				914			923			932				941				950	
V	P	Q	CAG	GTG	CCC	AGC	CCA	GGG	GGC	ACC	CCA	GGC	CAG	GCT	CCA	TAC	TAC	CCA	TAC	AGC	CTC
		965				974			983			992			1001						
P	E	P	A	L	A	L	A	L	D	S	S	G	K	N	L	T	E	Q	N	S	Y
CCT	GAG	CCT	GCC	CTC	CTC	GCC	CTG	GAC	TCT	AGC	GGG	AAG	AAC	AAC	CTG	ACA	GAG	CAG	AAC	AGC	TAC
		1025				1034			1043			1052			1061						
S	N	I	P	H	E	G	G	K	H	T	P	L	L	Y	E	R	S	S	P	I	N
AGC	AAC	ATT	CCT	CAC	GAG	GGG	AAG	CAC	ACA	CCG	CTG	TAT	GAG	CGC	TCC	TCG	CCC	ATC	AAC		
		1085				1094			1103			1112			1121						

FIG. 9E

L A Q S G S P N H V D S A Y F P G S T
 CTG GCC CAG AGC GGC AGC CCC AAT CAC GTG GAT TCC GCC TAC TTC CCT GGC TCG TCT ACC
 1145 1154 1163 1172 1181 1190

 S S S S D N D E G S G G A T N H I S G N
 TCG TCC TCA TCA GAC AAC GAT GAG GGC AGT GGA GGG GCA ACC AAC CAC ATC AGC GGC AAC
 1205 1214 1223 1232 1241 1250

 K I G W E K T G S H A E P L A R G D P G
 AAG ATT GGC TGG GAG AAG ACA GGA AGC CAT GCA GAG CCA CTG GCA CGG GGA GAC CCA GGA
 1265 1274 1283 1292 1301 1310

 D Q V K AAG GTA GAA GGT TCA TCC ACT GCC TCT TCG GGA AGC CAG CTA GCT GAA GGC
 1325 1334 1343 1352 1361 1370

 K G S H M G T V Q P I P C L L S M P T R
 AAA GGC AGC CAC ATG GGC ACT GTT CAG CCA ATC CCG TGC CTC CTG TCA ATG CCC ACC AGG
 1385 1394 1403 1412 1421 1430

FIG. 9F

N H M D I T T P P L P P P V A P E V L R V
 AAC CAC ATG GAT ATC ACC ACA CCT CCC TTG CCC CCC GTT GCA CCT GAG GTG CTG AGA GTG
 1445 1454 1463 1472 1481 1490

 A E H R H K K G L M Y P Y I F H V L T K
 GCT GAG CAC AGA CAC AAG AAG GGA CTG ATG TAC CCC TAC ATC TTC CAC GTC CTG ACA AAG
 1505 1514 1523 1532 1541 1550

 G E I K I A V S I E D E A N K D L P P A
 GGT GAA ATC AAA ATT GCT GTT TCT ATT GAA GAT GAA GGC AAC AAA GAC CTG CCT CCG GCC
 1565 1574 1583 1592 1601 1610

 A L L Y R P V R Q Y Y V Y G V L F S L A E
 GCC CTG CTC TAT AGG CCA GTT CGT CAG TAT GTT TAC GGA GTC CTG TTT AGT TTG GCA GAA
 1625 1634 1643 1652 1661 1670

 S R K K T E R L A F R K K N R L P P E F S
 AGC AGA AAG AAA ACT GAG AGA CTT GCT TTT AGA AAG AAC AGA CTT CCA CCA GAA TTC TCG
 1685 1694 1703 1712 1721 1730

FIG. 9G

P	L	I	I	K	E	W	A	A	Y	K	G	K	S	P	Q	T	P	E	L
CCA	CTG	ATC	ATT	AAG	GAA	TGG	GCA	GCT	TAC	AAG	GGG	AAG	TCT	CCT	CAG	ACC	CCG	GAA	CTA
					1754			1763			1772			1781			1790		
V	E	A	L	A	F	R	E	W	T	C	P	N	L	K	R	L	W	L	G
GTG	GAA	GCA	CTG	GCC	TTC	CGG	GAG	TGG	ACC	TGC	CCC	AAC	CTG	AAG	AGG	CTT	TGG	CTG	GGC
					1814			1823			1832			1841			1850		
K	A	V	E	D	K	N	R	R	M	R	A	F	L	A	C	M	R	S	D
AAG	GCG	GTG	GAG	GAC	AAG	AAC	CGC	CGG	ATG	AGG	GCC	TTC	CTG	GCC	TGC	ATG	AGG	TCT	GAT
					1874			1883			1892			1901			1910		
T	P	A	M	L	N	P	A	N	V	P	T	H	L	M	V	L	C	C	V
ACC	CCA	GCC	ATG	CTC	AAC	CCT	GCC	AAT	GTG	CCC	ACT	CAC	CTC	ATG	GTG	CTC	TGC	TGT	GTC
					1934			1943			1952			1961			1970		
L	R	Y	M	V	Q	W	P	G	A	R	I	L	R	R	Q	E	L	D	A
TTA	CGG	TAT	ATG	GTG	CAG	TGG	CCC	GGA	GCT	CGC	ATT	CTG	CGG	CGT	CAG	GAG	CTG	GAT	GCC
					1994			2003			2012			2021			2030		

FIG. 9H

F L A Q A L S P K L Y E P D Q L Q E L K
 TTC CTG GCT CAG GCA TTG TCT CTT CCC AAA CTC TAT GAG CCG GAC CAG CTG CAG GAA CTC AAG
 2045 2054 2063 2072 2081 2090

I E N L D P R G I Q L S A L F M S G V D
 ATT GAG AAC TTA GAC CCC CGA GGA ATT CAG CTG TCA GCT CTC TTC ATG AGC GGA GTA GAC
 2105 2114 2123 2132 2141 2150

M A L F A N D A C G Q P I P W E H C C P
 ATG GCA CTG TTT GCC AAT GAC GCG TGT GGT GGG CAG CCC ATA CCC TGG GAA CAC TGT TGT CCT
 2165 2174 2183 2192 2201 2210

W M Y F D G K L F Q S K K L L K A S R E K
 TGG ATG TAT TTC GAT GGG AAG CTC CTC TTT CTT AAA GCC AGC CGG GAA AAG
 2225 2234 2243 2252 2261 2270

T P L I D L C D G Q A E Q A A K V E K M
 ACC CCA CTC ATC GAC CTC TGT GAT GGT CAG GCT GAG CAG GCT GCC AAG GTT GAA AAG ATG
 2285 2294 2303 2312 2321 2330

FIG. 9I

R	Q	S	I	L	E	G	L	N	F	S	R	Q	N	H	P	L	P	F	P
CGC	CAG	AGT	ATC	CTG	GAG	GGG	CTC	AAC	TTC	TCC	CGG	CAA	AAC	CAC	CCA	CTC	CCC	TTC	CCA
		2345		2354				2363			2372			2381			2390		
P	P	P	A	L	P	F	Y	P	A	S	V	Y	P	R	H	F	G	P	V
CCA	CCT	CCT	GCT	CTG	CCC	TTC	TAC	CCA	GCT	TCT	GTA	TAT	CCT	CGG	CAC	TTT	GGG	CCG	GTC
		2405		2414				2423			2432			2441			2450		
P	P	S	Q	G	R	G	R	G	F	A	G	V	C	G	F	G	G	H	Y
CCA	CCC	TCA	CAG	GGC	AGG	GGC	AGG	GGT	TTT	GCA	GGA	GTC	TGT	GGC	TTT	GGA	GGC	CAT	TAT
		2465		2474				2483			2492			2501			2510		
G	E	T	V	A	T	G	P	Y	R	A	F	R	V	T	A	A	S	G	H
GGG	GAA	ACT	GTA	GCA	ACA	GGC	CCT	TAC	CGT	GCC	TTC	CGG	GTG	ACA	GCA	GCA	TCG	GGA	CAC
		2525		2534				2543			2552			2561			2570		
C	G	A	F	S	G	S	D	S	N	R	T	S	K	S	Q	G	G	V	Q
TGT	GGA	GCC	TTC	TCA	GGC	AGT	GAC	AGC	AAC	AGG	ACT	AGC	AAG	TCC	CAG	GGC	GGA	GTC	CAG
		2585		2594				2603			2612			2621			2630		

FIG. 9J

P I P S Q G G K L E I A G T V V G H W A
 CCT ATT CCT TCT CAG GGA GGG AAG CTA GAA ATA GCT GGC-ACG GTG GTC GGC CAC TGG GCT
 2645 2654 2663 2672 2681 2690

 G S R R G G R G G G R G G P F P L Q V S V G
 GGG AGC AGG CGG GGC CGT GGG GGC CGA GGC CCC TTC CCT CTG CAG GTG GTG TCT GTG GGA
 2705 2714 2723 2732 2741 2750

 G P A R G G R P R G V I S T P V I R T F G
 GGA CCA GCA AGA GGG CGT CCA AGG GGT GTT ATT TCC ACT CCT GTG ATT AGA ACA TTT GGA
 2765 2774 2783 2792 2801 2810

 R G G R Y Y G R G R G Y K S Q G A I Q G R P
 AGA GGT GGA AGG TAC TAC GGC AGA GGC TAT AAA AGC CAG GGA GCA ATT CAG GGC AGA CCT
 2825 2834 2843 2852 2861 2870

 P Y A A S A E E V A K E L K S K S G E S
 CCT TAT GCT GCT TCA GCA GAA GAA GGC AAA GAA CTG AAA TCG AAA TCT GGG GAA TCC
 2885 2894 2903 2912 2921 2930

FIG. 9K

K S S A V S S E G S L A E N G V M A E E
 AAG TCC TCT GCT GTG TCC TCA GAA GGG TCC CTG GCT GAA AAC GGA GTG ATG GCC GAG GAG
 2945 2954 2963 2972 2981 2990

 K P V P Q L N G S T A D T R A P S H S E
 AAG CCA GTG CCC CAA CTT AAT GGG AGC ACG GCT GAC ACC AGG GCC CCC AGC CAC TCT GAA
 3005 3014 3023 3032 3041 3050

 S A L N N D S K T C N T N P H L N A L S
 AGT GCC TTG AAC AAT GAC TCT AAA ACG TGC AAT ACA AAT CCT CAC TTA AAT GCA CTA AGT
 3065 3074 3083 3092 3101 3110

 T D S A C R R E A A L E A A V L N K E E
 ACA GAC AGC GCC TGC CGC AGG GAG GAG GCT GCT CTG GAG GCA GCT GTC TTA AAT AAA GAA GAG
 3125 3134 3143 3152 3161 3170

 TGA ACT TAT TTT TAT AGA GGG TGA AGG ATG CTG GAA GGG TAA GGA TTC AGG AAT ATC TGG
 3185 3194 3203 3212 3221 3230

FIG. 9L

AGA GAA AGA GAG CCT GCA GTT ATG TAC ATT TTG TCC TTT CCG TAA GAG AAA ATG AGG ACT
 3245 3254 3263 3272 3281 3290

GTG GAA ATT CAG ATC CCT CTT TGA TAT CAG AGA TTT AAA CAA CAC ATT CTT TTT TTA GTT
 3305 3314 3323 3332 3341 3350

TTA ACC AGT TGT AGT CAA AAT GCT ACA ATA AAA CAA AAC GAG AGA GAG AAA ATG AAG AGC
 3365 3374 3383 3392 3401 3410

ATT TGA CTC CCG CAC TTA AAA ATG AAG CAC ACA AAG TTT AAA CTG GTT ACG ACG ACA AAA
 3425 3434 3443 3452 3461 3470

FIG.9M

GCC TAC AGT TGT GTT TCT TGA ACT ATA AAG AAA ACA AAT TTT GGC AGT GTT TAA GTA TAT
 3485 3494 3503 3512 3521 3530

ATA GCT TAA AAT ATA ATT TTT AGC ATT TGG CAC CAT ATG TAT GCC ATT ATA TTT GAT TTT
 3545 3554 3563 3572 3581 3590

GCA TTA CTG TTT CAC AAT GAA GCT TTG TTT TAA GGC TTT GAT TTA TGA AAG AAA
 3605 3614 3623 3632 3641 3650

TAA GGC ACA ACC ACA GTT TTT CTT TCT TAC TTA AAT TTC ATC ACT GTT GAT GTG GTT CTT
 3665 3674 3683 3692 3701 3710

TTG TGA

FIG. 10A

Length of r84695-#85 like R405: 4680 bp; Listed from: 2 to: 4680;
Translated from: 152 to: 3505 (Entire region);
Genetic Code used: Universal;

[illegible]

FIG. 10B

G	S	L	V	G	G	G	G	R	P	P	Q	T	P	L	R	L	L	V
GGC	AGC	CTG	GTG	GGC	GGC	GGG	CGG	CGG	CCC	CCG	CAG	ACC	CCG	CTG	CGC	CTG	CTG	GTG
		244			253		262		271				280			289		
D	A	D	N	C	L	H	R	L	Y	G	G	F	T	D	W	V	S	G
GAC	GCC	GAC	AAC	TGC	CTG	CAC	CGC	CTC	TAC	GGC	GGC	TTC	TAC	ACC	GAC	TGG	GTC	AGC
		304			313			322			331			340		349		GGC
G	Q	W	N	H	M	L	G	Y	L	A	A	L	A	K	A	C	F	G
GGC	CAG	TGG	AAC	CAC	ATG	CTT	GGC	TAC	CTG	GGC	GGC	CTG	GCC	AAG	GCC	TGC	TTC	GGC
		364			373			382			391			400			409	GGC
N	I	E	L	F	V	F	F	N	G	A	L	E	K	A	R	L	H	E
AAC	ATC	GAG	CTC	TTC	GTC	TTC	TTC	AAC	GGC	GGC	CTC	GAG	AAG	GCC	CGG	CTG	CAC	GAG
		424			433			442			451			460			469	TGG
V	K	R	Q	G	N	E	R	Q	T	A	Q	Q	I	V	S	H	V	Q
GTC	AAG	CGG	CAG	GGC	AAC	GAG	GAG	CGC	CAG	ACG	GCA	CAG	ATC	GTC	AGC	CAT	GTC	CAG
		484			493			502			511			520			529	AAC

FIG.10C

K	G	T	P	P	P	K	V	W	F	L	P	P	P	V	C	M	A	H	C	I
AAG	GGC	ACC	CCG	CCG	CCA	AAG	GTC	TGG	TTC	CTG	CCG	CCC	GTC	TGC	ATG	GCC	CAC	TGC	ATC	
		544			553			562			571				580			589		
R	L	A	L	I	R	F	H	V	K	V	A	Q	S	I	E	D	H	H	Q	
CGC	CTG	GCG	CTC	ATC	CGC	TTC	CAC	GTC	AAG	GTT	GCA	CAG	AGC	ATT	GAG	GAT	CAC	CAT	CAG	
		604			613			622			631			640			649			
E	V	I	G	F	C	R	E	N	G	F	H	G	L	V	A	Y	D	S	D	
GAA	GTG	ATT	GGT	TTC	TGC	AGA	GAG	AAT	GGT	TTC	CAT	GGC	TTG	GTT	GCG	TAT	GAC	TCT	GAT	
		664			673			682			691			700			709			
Y	A	L	C	N	I	P	Y	Y	F	S	A	H	A	L	K	L	S	R	N	
TAT	GCA	CTG	TGC	AAC	ATC	CCC	TAC	TAT	TTC	AGT	GCC	CAT	GCC	CTA	AAA	CTG	AGC	CGG	AAC	
		724			733			742			751			760			769			
G	K	S	L	T	T	S	Q	Y	L	M	H	E	V	A	K	Q	L	D	L	
GGG	AAA	AGT	CTC	ACC	ACA	AGC	CAA	TAT	CTG	ATG	CAT	GAA	GTT	GCC	AAG	CAA	CTG	GAC	CTG	
		784			793			802			811			820			829			

109/183

FIG.10D

N	P	N	R	F	P	I	F	A	A	L	L	G	N	H	I	L	P	D	E
AAT	CCA	AAT	CGT	TTT	CCT	ATT	TTT	GCT	GCT	CTC	TTA	GGA	AAT	CAC	ATT	CTG	CCT	GAT	GAA
	844				853			862			871			880			889		
D	L	A	S	F	H	W	S	L	L	G	P	E	H	P	L	A	S	L	K
GAT	CTG	GCT	TCC	TTT	CAT	TGG	AGT	TTA	CTT	GGT	CCA	GAA	CAT	CCA	CTA	GCC	TCA	CTA	AAG
	904				913			922			931			940			949		
V	R	A	H	Q	L	V	L	P	P	C	D	V	V	I	K	A	V	A	D
GTC	CGG	GCC	CAC	CAG	CTG	GTC	TTG	CCA	CCT	TGC	GAC	GTA	GTG	ATC	AAA	GCC	GTT	GCT	GAC
	964				973			982			991			1000			1009		
Y	V	R	N	I	Q	D	T	S	D	L	D	A	I	A	K	D	V	F	Q
TAT	GTA	CGC	AAC	ATT	CAG	GAC	ACC	TCT	GAC	TTG	GAT	GCC	ATA	GCT	AAA	GAT	GTT	TTC	CAG
	1024				1033			1042			1051			1060			1069		
H	S	Q	S	R	T	D	D	K	V	I	R	F	K	R	A	I	G	Y	Y
CAT	TCA	CAG	TCT	AGA	ACA	GAT	GAC	AAA	GTT	ATT	CGA	TTT	AAG	AGA	GCA	ATT	GGA	TAT	TAT
	1084				1093			1102			1111			1120			1129		

FIG.10E

S A T S K P M S F H P P H Y L A A R P G
 TCA GCG ACT AGT AAG CCT ATG TCA TTT CAT CCA CCA CAT TAC TTA GCA GCC AGA CCC GGT
 1144 1153 1162 1171 1180 1189

 P F G M P G M V P P P H V P P Q M L N I P
 CCG TTT GGA ATG CCT GGG ATG GTG CCG CCG CAT GTT CCT CCT CAG ATG CTC AAC ATT CCG
 1204 1213 1222 1231 1240 1249

 Q T S L Q A K P V A P Q V P S P G G A P
 CAG ACC TCT CTG CAA GCA AAG CCC GTG GCC CCA CAG GTG CCC AGC CCA GGG GGC GCC CCG
 1264 1273 1282 1291 1300 1309

 G Q G P Y P Y S L S E P A P L T L D T S
 GGC CAG GGT CCA TAC CCG TAC AGC CTC TCT GAG CCA GCA CCT CTC ACT TTG GAC ACG AGC
 1324 1333 1342 1351 1360 1369

 G K N L T E Q N S Y S N I P H E G K H T
 GGG AAG AAT CTG ACG GAG CAG AAC AGC TAC AGC AAC ATT CCT CAC GAA GGG AAG CAC ACG
 1384 1393 1402 1411 1420 1429

111/183

FIG. 10F

P L Y E R S S P I N R A Q S G S P N H V
 CCG C'IG TAT' GAG CGG TCC TCG CCC ATC AAC CGG GCC CAG AGC GGC AGC CCC AAC CAC GTG
 1444 1453 1462 1471 1480 1489

 D S A Y F P G S S T S S S D N D E G S
 GAT TCC GCC TAC TTC CCT GGC TCT TCT ACA TCG TCA TCT TCC TCC GAC AAC GAC GAG GGC AGC
 1504 1513 1522 1531 1540 1549

 G G A T N H I S G N K I G W E K T G S H
 GGA GGG GCG ACA AAC CAT ATC AGC GGG AAC AAG AAG ATT GGC TGG GAG AAG ACG GGA AGC CAC
 1564 1573 1582 1591 1600 1609

 S E P Q A R G G D P G D Q T K A E G S S T
 TCA GAG CCT CAG GCA CGA GGA GAC CCA CGA GAC CAA ACA AAG GCA GAA GGC TCG TCC ACT
 1624 1633 1642 1651 1660 1669

 A S S G S Q L A E G K G G G AAG GGA AGC CAG ATG GGC ACT GTC CAG CCA
 GCC TCT TCA GGA AGC CAA CTA GCC GAA GAA GGC AAG GGC ACT GTC CAG CCA
 1684 1693 1702 1711 1720 1729

112/183

FIG.10G

I	P	C	L	L	S	M	P	T	R	N	H	M	D	I	T	T	P	P	L
ATC	CCG	TGC	CTC	CTG	TCG	ATG	CCC	ACC	AGG	AAC	CAC	ATG	GAC	ATC	ACC	ACA	CCT	CCC	CTG
		1744			1753		1762				1771			1780			1789		
P	P	V	A	P	E	V	L	R	V	A	E	H	R	H	K	K	G	L	M
CCC	CCC	GTC	GCA	CCT	GAG	GAG	GTG	CTG	AGA	GTG	GCC	GAG	CAC	AGG	CAC	AAG	AAG	GGG	CTG
		1804			1813		1822				1831			1840			1849		
Y	P	Y	I	F	H	V	L	T	K	G	E	I	K	I	A	V	S	I	E
TAC	CCC	TAC	ATC	TTC	CAT	GTC	CTG	ACG	AAG	GGT	GAA	ATC	AAA	ATT	GCT	GTT	TCT	ATT	GAA
		1864			1873		1882				1891			1900			1909		
D	E	A	N	K	D	L	P	P	A	A	L	L	Y	R	P	V	R	Q	Y
GAT	GAA	GCC	AAC	AAG	GAC	CTG	CCT	CCG	GCC	GCT	CTG	CTC	TAT	AGG	CCA	GTT	CGT	CAG	TAT
		1924			1933		1942				1951			1960			1969		
V	Y	G	V	L	F	S	L	A	E	S	R	K	K	T	E	R	L	A	F
GTT	TAC	GGA	GTC	CTG	TTT	AGT	TTG	GCA	GAA	AGC	AGA	AAG	AAA	ACT	GAG	AGA	CTT	GCT	TTT
		1984			1993		2002				2011			2020			2029		

FIG. 10H

R K N R L P P P E F S P V I I K E W A A Y
 AGA AAG AAC AGA CTT CCA CCA GAA TTT TCA CCA GTG ATC ATT AAA GAA TGG GCA GCT TAC
 2044 2053 2062 2071 2080 2089

 K G K S P Q T P E L L V E A L A F R E W T
 AAA GGA AAG TCT CCT CAA ACC CCG GAA CTG GTT GAA GCT CTT GCC TTC AGG GAG TGG ACC
 2104 2113 2122 2131 2140 2149

 C P N L K R L W L G K A V E D K N R R M
 TGC CCC AAC CTG AAG AGG CTG TGG TTG GGT AAG GCG GTA GAG GAC AAC AAC CGC AGG ATG
 2164 2173 2182 2191 2200 2209

 R A F L A C M R S D T P A M L N P A N V
 AGG GCC TTC CTG GCC TGC ATG AGG TCG GAC ACC CCA GCC ATG CTC AAC CCT GCC AAC GTG
 2224 2233 2242 2251 2260 2269

 P T H L M V L C C V L R Y M V Q W P G A
 CCC ACT CAC CTC ATG GTG CTC TGC TGC GTC TTA CGG TAC ATG GTG CAG TGG CCG GGA GCA
 2284 2293 2302 2311 2320 2329

FIG. 10 I

C	I	L	R	R	Q	E	L	D	A	F	L	A	Q	A	L	S	P	K	L
TGC	ATC	CIT	CGG	CGT	CAG	GAG	CIA	GAT	GCC	TTC	CTG	GCT	CAG	GCG	CTG	TCC	CCC	AAA	CTC
		2344			2353			2362					2371		2380			2389	
Y	E	P	D	Q	L	Q	E	L	K	I	E	N	L	D	P	R	G	I	Q
TAC	GAG	CCT	GAT	CAG	CTC	CAG	GAG	CTC	AAG	ATT	GAG	AAC	CTA	GAT	CCC	CGA	GGA	ATT	CAG
		2404			2413			2422					2431		2440			2449	
L	S	A	L	F	M	S	G	V	D	M	A	L	F	A	N	D	A	C	G
CTA	TCA	GCT	CTC	TTC	ATG	AGT	GGA	GTA	GAC	ATG	GCC	TTG	TTT	GCA	AAT	GAT	GCA	TGC	GGA
		2464			2473			2482					2491		2500			2509	
Q	P	I	P	W	E	H	C	C	P	W	M	Y	F	D	G	K	L	F	Q
CAG	CCA	ATC	CCC	TGG	GAA	CAC	TGT	TGT	CCT	TGG	ATG	TAT	TTT	GAT	GGG	AAG	CTC	TTC	CAA
		2524			2533			2542					2551		2560			2569	
S	K	L	L	K	A	S	R	E	K	T	P	L	I	D	L	C	D	G	Q
TCC	AAA	CTC	CTC	AAA	GCC	AGC	CGG	GAA	AAG	ACC	CCA	CTC	ATT	GAC	CTC	TGT	GAT	GGT	CAG
		2584			2593			2602				2611		2620				2629	

115/183

FIG. 10J

A	D	Q	A	A	K	V	E	K	M	R	Q	S	V	L	E	G	L	S	F
GCT	GAT	CAG	GCT	GCC	AAG	GTA	GAG	AAG	ATG	CGC	CAG	AGC	GTC	CTC	GAG	GGG	CTC	AGC	TTC
		2644			2653			2662			2671			2680			2689		
S	R	Q	S	H	T	L	P	F	P	P	P	P	A	L	P	F	Y	P	A
TCC	AGG	CAG	ACC	CAC	ACG	CTC	CCT	TTC	CCG	CCG	CCA	CCT	GCC	CTG	CCC	TTC	TAC	CCT	GCC
		2704			2713			2722			2731			2740			2749		
S	A	Y	P	R	H	F	G	P	V	P	P	S	Q	G	R	G	R	G	F
TCT	GCG	TAC	CCC	CGG	CAC	TTT	GGG	CCT	GTC	CCA	CCC	TCT	CAG	GGC	AGG	GGC	AGA	GGC	TTT
		2764			2773			2782			2791			2800			2809		
A	G	V	C	G	F	G	G	P	Y	G	E	T	V	A	T	G	P	Y	R
GCA	GGC	GTC	TGT	GGC	TTT	GGA	GGC	CCC	TAT	GGG	GAA	ACG	GTA	GCA	ACA	GGC	CCT	TAC	CGT
		2824			2833			2842			2851			2860			2869		
A	F	R	V	A	A	A	S	G	H	C	G	A	F	S	G	S	D	S	S
GCC	TTC	CGT	GTG	GCG	GCA	GCA	TCG	GGA	CAC	TGC	GGA	GCC	TTC	TCA	GGC	AGT	GAC	AGC	AGC
		2884			2893			2902			2911			2920			2929		

116/183

FIG.10K

R T S K S Q G G V Q P I P S Q Q G G K L E
 AGG ACT AGC AAG TCC CAG GGC GGA GTC CAA CCT ATA CCT TCT CAG GGA GGC AAA CTA GAA
 2944 2953 2962 2971 2980 2989

 I A G T V V G G H W A G S R R G R G R G
 ATA GCT GGC ACT GTG GTT GGC CAT TGG GCT GGG AGC AGG CGG GGC CGT GGC CGG GGC
 3004 3013 3022 3031 3040 3049

 P F P L Q V V S V G G P A R G R P R G V
 CCT TTC CCC CTG CAG GTG GTT TCT GTC GGA GGA CCA GCT AGA GGG CGT CCA AGA GGA GTT
 3064 3073 3082 3091 3100 3109

 I S T P V I R T F G R G G R Y G R G Y
 ATT TCC ACC CCA GTG ATT AGA ACA TTT GGA AGA GGT GGA AGG TAC TAT GGC AGA GGT TAC
 3124 3133 3142 3151 3160 3169

 K N Q A A I Q G R P P Y A A S A E E V A
 AAA AAC CAG GCA GCA ATT CAG GGC AGA CCT CCT TAT GCT GCT TCA GCA GAA GAA GTG GCC
 3184 3193 3202 3211 3220 3229

117/183

FIG.10L

K E L K S K S G E S K S S A M S S D G S
 AAA GAA CTT AAG TCA AAA TCT GGG GAA TCG AAG TCC TCT GCT ATG TCT TCA GAC GGG TCC
 3244 3253 3262 3271 3280 3289

 L A E N G V M A E E K P A P Q M N G S T
 CTG GCT GAA AAC GGA GTG ATG GCC GAG GAG AAG CCG GCT CCC CAG ATG AAC GGG AGC ACG
 3304 3313 3322 3331 3340 3349

 G D A R A P S H S E S A L N N D S K T C
 GGT GAC GCC AGG GCC CCC AGC CAC TCT GAA AGT GCC TTG AAT AAT GAC TCT AAA ACG TGC
 3364 3373 3382 3391 3400 3409

 N T N P H L N A L S T D S A C R R E A A
 AAT ACA AAT CCT CAT TTA AAT GCA CTA AGT ACA GAC AGC GCT TGC CGC AGA GAA GCT GCT
 3424 3433 3442 3451 3460 3469

 L E A A V L N K E E
 CTG GAG GCA GCT GTC TTA AAT AAA GAA GAG TAA ACT TAT TTT TTA TAG AGG GTG AAG GAT
 3484 3493 3502 3511 3520 3529

FIG.10M

GCT GGA AGG GTA AGG ATT TAG GAA TAT CTG GAG AGA AAG AGA GCC TGC AGT TAT GTA CAT
 3544 3553 3562 3571 3580 3589

 TTT GTC CTT TCC GTA AGA GAA AAA TGA GGA CTT TGG AAA TTC AGA TCC CTC TTT GAT ATC
 3604 3613 3622 3631 3640 3649

 AGA GAT TTA AAC AAC ACA TTT TTA GTT TTA ACC AGT TGT AGT CAA AAT GCT ACA ATA AAA
 3664 3673 3682 3691 3700 3709

 CAA AAA AGA GAA AGA AAA TGA AGA GCA TTT GAC TCC CGC ACT TAA AAT GAA GTA CAC ATA
 3724 3733 3742 3751 3760 3769

 AAG TTT AAA CTG GTT ATG ACA AAA GCC TAT AGT TGT GTT TCT TGA ACT ATA AAG AAA ACA
 3784 3793 3802 3811 3820 3829

FIG. 10N

AAT TTT GGC AGT CTT TAA GTA TAT ATA GCT TAA AAT ATA ATT TTT AGC ATT TGG CAC CAT	3844	3853	3862	3871	3880	3889
ATG TAT GCC ATT ATA TTT GAT TTT GCA TTA CTG TTT CAC AAT GAA GCT TTC TTT AAG GCT	3904	3913	3922	3931	3940	3949
TTG ATT TTT ATG ATT ATG AAA GAA ATA AGG CAC AAC CAC AGT TTT TCT TTA AAT TTC	3964	3973	3982	3991	4000	4009
ATC ACT GTT GAT GTG GTT CTT TTG TGT TAA AAA AAA AAA GTG CAA CTA TCA AAA CTA AAA	4024	4033	4042	4051	4060	4069
AAT TAT AGA GTA ATA TTG CCG TTC TGC TGA TTT TAA ATA TAC AAT ACA TCA TAC ATA CTT	4084	4093	4102	4111	4120	4129

120/183

FIG.10 0

TAC AAG CAA GTT AAA TGG AGA TAA AGT TGA AAT CAT AGA AGA TGC AAA TGA CCT TTC AAA
 4144 4153 4162 4171 4180 4189

 ATC AAC ACA ATG TGT TCT GAA ACT TTC GTG ACT AAT ACC ATG CAT CTG TGA TCA ATG AAC
 4204 4213 4222 4231 4240 4249

 TAT GTG GTT TTG AAT CGG ATG TAG ACC ATT AGT ACT ACT TGA GCT AAA CTT CTG CAT
 4264 4273 4282 4291 4300 4309

 GGT TCA TAA TTT TTA AAG TGT GTA GTT AAT ATG CAT GTT ATC GTC CTT TCT TCC ATT CTT
 4324 4333 4342 4351 4360 4369

 AAC AGT ATG TGC CCA TTT GCA AAA CAA AAA TGC TAA TAA TCA GTA ATA GTC CTA TAA AAG
 4384 4393 4402 4411 4420 4429

FIG. 10P

ATG TTA ACT CTG TTT AGT CAT TGA CTG ATC TTG CTC TAA CCT TAA AAT TTT GTG ATT ATT	4444	4453	4462	4471	4480	4489
GAC CTC TGT TGC ATT TAT TCT AAA GCC CCC CAA AAA TTA TCT AGC CGT TTC GAA TAT CAA	4504	4513	4522	4531	4540	4549
CAT TAC CCT GGT GTA TTC ACT GCT GTA TGC ATT ATT GTT CTT TGT TGC TGT TTT ATG CCT	4564	4573	4582	4591	4600	4609
TCA TAT TAG CAA ATA TGA AAT TCT GTG AAA AAA AAC CCT TTG ATC TTA AAA AAA AAA AAA	4624	4633	4642	4651	4660	4669
AAA AA						

FIG. IIA

405: CLUSTAL X (1.64b) Multiple Sequence Alignment

```

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166
-----
MGVQGFQDYIEKHCP SAVVPVELQKLARGLVGGGRQRPQTPLRLLVDA
-----
--DYIEKHCP SAVVPVELQKLARGLVGGGRQRPQTPLRLLVDA
-----

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166
-----
--MLGYLAALAKACFGGNIELFVFFNGAL
-----
DNCLHRLYGGFYTDWVSGGQWNHMLGYLAALAKACFGGNIELFVFFNGAL
DNCLHRLYGGFYTDWVSGGQWNHMLGYLAALAKACFGGNIELFVFFNGAL
-----

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166
-----
EKARLHEWVKRQGNERTAQQIVSHVQNKGTPPPKNWFLPPVCMAMHCIRL
-----
EKARLHEWVKRQGNERTAQQIVSHVQNKGTPPPKNWFLPPVCMAMHCIRL
EKARLHEWVKRQGNERTAQQIVSHVQNKGTPPPKNWFLPPVCMAMHCIRL
-----

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166
-----
ALIRFHVKVAQSIEDHHQEVIGFCRENGEHLVAYDSYALCNIPYYFSA
-----
ALIRFHVKVAQSIEDHHQEVIGFCRENGEHLVAYDSYALCNIPYYFSA
ALIRFHVKVAQSIEDHHQEVIGFCRENGEHLVAYDSYALCNIPYYFSA
-----

```

FIG. 11B

405: CLUSTAL X (1.64b) Multiple Sequence Alignment

405_no_up	HALKLSRNGKSLTTSQYLMHEVAKQLDLPNRRFPIFAALLGNHILPDEDL
gi 3005744	-----
405_hum	HALKLSRNGKSLTTSQYLMHEVAKQLDLPNRRFPIFAALLGNHILPDEDL
gi 1136426 gnl PID d1012166	HALKLSRNGKSLTTSQYLMHEVAKQLDLPNRRFPIFAALLGNHILPDEDL
405_no_up	ASFHWSLLGPEHPLASLKVRAHQLVLPCCDVVIKAVADYVRNIHDTSDLD
gi 3005744	-----
405_hum	ASFHWSLLGPEHPLASLKVRAHQLVLPCCDVVIKAVADYVRNIQDTSDDL
gi 1136426 gnl PID d1012166	ASFHWSLLGPEHPLASLKVRAHQLVLPCCDVVIKAVADYVRNIQDTSDDL
405_no_up	AIAKDVFOHSQSRTDDKVIREFKRAVGYYSATSKPMPFHPHYLPR-PNPF
gi 3005744	-----
405_hum	AIAKDVFOHSQSRTDDKVIREFKRAIGYYSATSKPMSFHPHYLAARPGPF
gi 1136426 gnl PID d1012166	AIAKDVFOHSQSRTDDKVIREFKRAIGYYSATSKPMSFHPHYLAARPGPF
405_no_up	GMPGMVPPYVPPQMLNIPQTSLOAKEAVPQVPSGGTGGQAPYPYSLPEP
gi 3005744	-----
405_hum	GMPGMVPPHVPVPPQMLNIPQTSLOAKEVAPQVPSGGAPGGQGPYPYSLSEP
gi 1136426 gnl PID d1012166	GMPGMVPPHVPVPPQMLNIPQTSLOAKEVAPQVPSGGAPGGQGPYPYSLSEP

FIG. 11C

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166

A-LALDSSGKNLTEQNSYSNI PHEGKHTPLYERSSPINLAQSGSPNHVDS

APLTLDTSCKNLTEQNSYSNI PHEGKHTPLYERSSPINRAQSGSPNHVDS
APLTLDTSCKNLTEQNSYSNI PHEGKHTPLYERSSPINPAQSGSPNHVDS

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166

AYFPGSSTSSSDNDEGSGGATNHI SGNKIGWEKTGSHAEP LARGDPGDQ

AYFPGSSTSSSDNDEGSGGATNHI SGNKIGWEKTGSHSEPOARGDPGDQ
AYFPGSSTSSSDNDEGSGGATNHI SGNKIGWEKTGSHSEPOARGDPGDQ

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166

VKVEGSSTASSGSQLAEGKSGHMGTVQPI PCLLSMPTRNHMDITTPPLPP

TKAEGSSTASSGSQLAEGKSGMQGTVQPI PCLLSMPTRNHMDITTPPLPP
TKAEGSSTASSGSQLAEGKSGMQGTVQPI PCLLSMPTRNHMDITTPPLPP

405 no up
gi|3005744
405 hum
gi|1136426|gnl|PID|d1012166

VAPEVL RVAEHRHKGLMYPYIFHVLTKEIKIAVSIEDEANKDLPPAAL
-----GEIKIAVSIEDEANKDLPPAAL
VAPEVL RVAEHRHKGLMYPYIFHVLTKEIKIAVSIEDEANKDLPPAAL
VAPEVL RVAEHRHKGLMYPYIFHVLTKEIKIAVSIEDEANKDLPPAAL

FIG. 11D

405_no_up
gi|3005744
405_hum
gi|1136426|gnl|PID|d1012166

LYRPVRQYVYGVLFSLAESRKKTERLAFRKNRLPPEFSPVLIKEWAAAYKG
LYRPVRQYVYGVLFSLAESRKKTERLAFRKNRLPPEFSPVLIKEWAAAYKG
LYRPVRQYVYGVLFSLAESRKKTERLAFRKNRLPPEFSPVLIKEWAAAYKG
LYRPVRQYVYGVLFSLAESRKKTERLAFRKNRLPPEFSPVLIKEWAAAYKG
*****:*****

405_no_up
gi|3005744
405_hum
gi|1136426|gnl|PID|d1012166

KSPQTPELVEALAFREWTCPNLKRLLWLGKAVEDKNRRMRAFLACMRSDTP
KSPQTPELVEALAFREWTCPNLKRLLWLGKAVEDKNRRMRAFLACMRSDTP
KSPQTPELVEALAFREWTCPNLKRLLWLGKAVEDKNRRMRAFLACMRSDTP
KSPQTPELVEALAFREWTCPNLKRLLWLGKAVEDKNRRMRAFLACMRSDTP

405_no_up
gi|3005744
405_hum
gi|1136426|gnl|PID|d1012166

AMLNPANVPTHLMVLCVLRVMVQWPGARILRRQELDAFLAQALSPKLYE
AMLNPANVPTHLMVLCVLRVMVQWPGARILRRQELDAFLAQALSPKLYE
AMLNPANVPTHLMVLCVLRVMVQWPGACILRRQELDAFLAQALSPKLYE
AMLNPANVPTHLMVLCVLRVMVQWPGARILRRQELDAFLAQALSPKLYE

405_no_up
gi|3005744
405_hum
gi|1136426|gnl|PID|d1012166

PDQLQELKIENLDPRGILSALFMSGVDMALFANDACGQPIPWEHCCPWW
PDQLQELKIENLDPRGILSALFMSGVDMALFANDACGQPIPWEHCCPWW
PDQLQELKIENLDPRGILSALFMSGVDMALFANDACGQPIPWEHCCPWW
PDQLQELKIENLDPRGILSALFMSGVDMALFANDACGQPIPWEHCCPWW

[illegible]

FIG. IIF

G R Y Y G R G Y K S Q G A I Q G R P P Y A A S A E E V A K E L K S K S G E S K S S A V S S E G S L A
 G R Y Y G R G Y K N Q A A I Q G R P P Y A A S A E E V A K E L K S K S G E S K S S A M S S D G S L A
 G R Y Y G R G Y K N Q A A I Q G R P P Y A A S A E E V A K E L K S K S G E S K S S A M S S D G S L A
 G R Y Y G R G Y K N Q A A I Q G R P P Y A A S A E E V A K E L K S K S G E S K S S A M S S D G S L A
 ***** * . ***** : *** : *****

405_no_up
 gi|3005744
 405_hum
 gi|1136426|gnl|PID|d1012166

E N G V M A E E K F V P Q I N G S T A D T R A P S H S E S A L N N D S K T C N T N P H L N A L S T D
 E N G V M A E E K P A P O M N G S T G D A R A P S H S E S A L N N D S K T C N T N P H L N A L S T D
 E N G V M A E E K P A P O M N G S T G D A R A P S H S E S A L N N D S K T C N T N P H L N A L S T D
 E N G V I G R ----- G E A G S P D E R E H G -----
 ***** : * . : * . . *

405_no_up
 gi|3005744
 405_hum
 gi|1136426|gnl|PID|d1012166

S A C R R E A A L E A A V L N K E E
 S A C R R E A A L E A A V L N K E E
 S A C R R E A A L E A A V L N K E E

405_no_up
 gi|3005744
 405_hum
 gi|1136426|gnl|PID|d1012166

274 Human

Probe:

Pool of probes
representing a 8Kb
of the 274 Human
contig

Target: (total RNA
sources)

1. Rat Bone
2. Rat Testis
3. Human cell line
NB4

M 1 2 3

*FIG. 12*

FIG.13A

Length of 274 25.3.99: 10427 bp; Listed from: 1 to: 10427;
Translated from: 211 to: 9996 (Entire region);
Genetic Code used: Universal;

Frame 1

CGA	CAA	AGG	CAG	CCT	GTG	CCG	CAC	AGT	TGA	GGG	GTG	CCG	TGA	GGA	GCT	GCA	GAA	54		
		9		18		27		36				45								
TCA	GGC	CAA	TTT	CTC	CTT	CGC	TCC	TCT	CGT	GTT	AGA	CAT	GCT	CAA	TTT	CCT	CAT	GGA	TGC	108
		63		72		81		90						99						
CAT	TCA	GAC	CAA	CTT	TCA	CAG	GCT	TCC	GCC	GTG	GGG	AGC	AGC	AGC	CGG	GCA	CAG	CAG	GCC	168
		123		132		141		150						159						
OPEN																		←		
CTC	AGT	GAA	CTG	CAC	ACA	GTG	GAC	AAG	GTT	GTG	GAG	ATG	ACA	GAC	CAG	CTG	ATG	GTT	CCC	228
		183		192		201		210						219						

FIG. 13B

T	L	G	S	Q	E	G	A	F	E	N	V	R	M	N	Y	S	G	D	Q
ACC	TTA	GGC	TCA	CAG	GAA	GGT	GCC	TTT	GAG	AAT	GTT	CGG	ATG	AAT	TAC	AGC	GGA	GAC	CAG
		243			252			261			270			279			288		
G	Q	CAG	ACT	ATA	CGG	CAG	CTG	ATC	AGT	GCC	CAC	GTG	CTC	AGG	CGG	GTG	GCT	ATG	TGT
		303			312			321				330			339			348	
L	S	S	P	H	G	R	R	Q	H	L	A	V	S	S	H	E	K	G	I
CTT	TCT	TCC	CCC	CAT	GGG	CGC	CGC	CAA	CAT	TTG	GCT	GTC	AGC	CAC	GAG	AAG	GGC	AAG	ATC
		363			372			381				390		399			408		
T	V	L	Q	L	S	A	L	L	K	Q	A	D	S	S	S	K	R	K	L
ACG	GTG	CTG	CAG	CTC	TCG	GCG	CTC	CTG	AAG	CAA	GCA	GAT	TCC	AGT	AAG	AGG	AAG	TTG	ACT
		423			432			441			450			459			468		
L	T	R	L	A	S	A	P	V	P	F	T	V	L	S	L	T	G	N	P
CTA	ACC	CGC	TTG	GCT	TCT	GCT	CCA	GTT	CCC	TTC	ACC	GTG	CTG	AGT	CTT	ACT	GGA	AAC	CCC
		483			492			501			510			519			528		

C K E D Y L X V C G L K A H V L T F S
 TGC AAG GAG GAC TAC CTG SCA GTG TGT GGG CTG AAG GAC TGC CAT GTG CTC ACC TTC AGT
 543 552 561 570 579 588

 S S G S V S D H L V L H P Q L A T G N F
 AGC TCG GGC TCT GTC TCC GAT CAC TTA GTG TTA CAT CCC CAG TTG GCA ACA GGG AAC TTC
 603 612 621 630 639 648

 I I K A V W L P G S Q T E L A I V T A D
 ATC ATC AAA GCT GTG TGG CTC CCT GGC TCT CAG ACC GAG CTA GCC ATT GTT ACT GCA GAC
 663 672 681 690 699 708

 F V K I Y D L S V D A L S P T F Y F L L
 TTC GTC AAG ATT TAC GAC CTG TCT GTT GAT GCC TTG AGC CCC ACC TTC TAC TTC CTC CTG
 723 732 741 750 759 768

 P S S K I R D V T F L F N E E G K N I I
 CCA AGC TCA AAG ATA AGG GAT GTC ACC TTC CTT TTC AAC GAG GAA GGC AAG AAC ATC ATT
 783 792 801 810 819 828

FIG.13C

132/183

FIG. 13D

V	I	M	S	S	A	G	Y	M	Y	T	Q	L	M	E	E	A	S	S	A
GTC	ATC	ATG	TCC	TCT	GCT	GGG	TAC	ATG	TAC	ACA	CAG	CTC	ATG	GAG	GAG	GCC	AGC	AGT	GCC
		843			852		861				870			879			888		
Q	Q	G	P	F	Y	V	T	N	V	L	E	I	N	H	E	D	L	K	D
CAG	CAG	GGG	CCC	TTC	TAT	GTC	ACT	AAT	GTG	CTG	GAA	ATA	AAT	CAC	GAG	GAT	CTG	AAG	GAC
		903			912		921				930			939			948		
S	N	S	Q	V	A	G	G	G	V	S	V	Y	Y	S	H	V	L	Q	M
AGT	AAC	AGC	CAG	GTG	GCA	GGT	GGT	GGC	GTG	TCT	GTG	TAC	TAT	TCA	CAC	GTG	TTG	CAG	ATG
		963			972		981				990			999			1008		
L	F	F	S	Y	S	Q	G	G	S	F	A	A	T	V	S	R	S	T	L
CTT	TTC	TTC	AGC	TAC	AGT	CAG	GGC	AAG	TCC	TTT	GCA	GCC	ACC	GTC	AGC	AGG	AGC	ACT	CTG
		1023			1032		1041				1050			1059			1068		
E	V	L	Q	L	F	P	I	N	I	K	S	S	N	G	G	S	K	T	S
GAG	GTG	CTG	CAG	CTC	TTC	CCC	ATC	AAC	ATC	AAA	AGC	TCC	AAT	GGT	GGC	AGT	AAG	ACT	TCT
		1083			1092		1101				1110			1119			1128		

133/183

FIG. 13E

P	A	L	C	Q	W	S	E	V	M	N	H	P	G	L	V	C	C	V	Q
CCC	GCC	CTT	TGC	CAG	TGG	TCT	GAG	GTG	ATG	AAC	CAC	CCT	GGC	TTG	GTG	TGT	TGT	GTT	CAG
		1143			1152			1161			1170			1179				1188	
Q	T	T	G	V	P	L	V	V	M	V	K	P	D	T	F	L	I	Q	E
CAA	ACT	ACT	GGT	GTG	CCT	CTG	GTA	GTC	ATG	GTG	AAA	CCA	GAC	ACT	TTC	CTC	ATC	CAG	GAG
		1203			1212			1221			1230			1239				1248	
I	K	T	L	P	A	K	A	K	I	Q	D	M	V	A	I	R	H	T	A
ATT	AAG	ACT	CTT	CCC	GCC	AAA	GCA	AAG	ATC	CAG	GAC	ATG	GTT	GCT	ATT	AGA	CAC	ACT	GCC
		1263			1272			1281			1290			1299				1308	
C	N	E	Q	Q	R	T	T	M	I	L	L	C	E	D	G	S	L	R	I
TGT	AAT	GAG	CAG	CAG	CGC	ACC	ACC	ATG	ATC	CTG	CTG	TGT	GAG	GAT	GGC	AGC	CTG	CGA	ATT
		1323			1332			1341			1350			1359				1368	
Y	M	A	N	V	E	N	T	S	Y	W	L	Q	P	S	L	Q	P	S	S
TAT	ATG	GCC	AAC	GTG	GAG	AAC	ACC	TCT	TAC	TGG	CTC	CAG	CCG	TCT	CTG	CAG	CCC	AGC	AGC
		1383			1392			1401			1410			1419				1428	

134/183

FIG. 13F

V I S I M K P V R K R K T A T I T A R T
 GTC ATC AGC ATC ATG AAG CCT GTG CGA AAG CGC AAA ACA GCT ACA ATC ACA GCC CGC ACA
 1443 1452 1461 1470 1479 1488

 S S Q CAG GTG ACC TTC CCC ATT GAC TTC TTT GAA CAC AAC CAG CAG CTA ACG GAT GTG
 TCC AGC CAG GTG ACC TTC CCC ATT GAC TTC TTT GAA CAC AAC CAG CAG CTA ACG GAT GTG
 1503 1512 1521 1530 1539 1548

 E F G G N D L L Q V Y N A Q Q I K H R L
 GAG TTT GGT AAT GAC CTC CTG CAA GTC TAC AAT GCG CAA CAG ATA AAG CAC AGG CTC
 1563 1572 1581 1590 1599 1608

 N S T G M Y V A N T K P G G G F T M E I S
 AAC TCC ACT GGC ATG TAT GTG GCG AAC ACC AAG CCT GCA GGC TTC ACC ATG GAG ATC AGT
 1623 1632 1641 1650 1659 1668

 N N S S T M V M T G M R I Q I G T Q A I
 AAC AAC AGT AGC ACC ATG GTG ATG ACG GGC ATG CGG ATC CAG ATT GGC ACA CAG GCA ATC
 1683 1692 1701 1710 1719 1728

135/183

FIG. 13G

E R A P S Y I E I F G R T M Q L N L S R	
GAG CGA GCA CCG TCC TAC ATC GAG ATC TTT GGC AGA ACC ATG CAG CTT AAC CTG AGC CGC	1743 1752 1761 1770 1779 1788
S R W F D F P P F T R E E A L Q A D R K L	
TCC CGC TGG TTT GAC TTC CCC TTC ACC AGA GAG GAA GCC CTG CAG GCT GAC CGG AAG CTG	1803 1812 1821 1830 1839 1848
N L F I G A S V D P A G V T M I D A V K	
AAC CTC TTC ATC GGT GCC TCT GTG GAT CCA GCT GGC GTC ACC ATG ATA GAT GCT GTA AAA	1863 1872 1881 1890 1899 1908
I Y G K T K E Q F G W P D E P P E D F P	
ATT TAT GGC AAG ACT AAA GAG CAG TTT GGC TGG CCT GAT GAG CCC CCA GAA GAC TTC CCT	1923 1932 1941 1950 1959 1968
S A S V S S V C P P N L N Q S N G T G D	
TCT GCC TCT GTT AGC AGC GTC TGC CCT CCT AAC CTG AAC CAG AGC AAC GGC ACT GGA GAC	1983 1992 2001 2010 2019 2028

FIG. 13H

S D S A A P A T T S G T V L E R L V S
 AGT GAC TCA GCT GCT CCA GCT ACA ACC AGT GGC ACT GTC CTG GAG AGG CTG GTT GTG AGT
 2043 2052 2061 2070 2079 2088

 S L E A A L E S C F A V G G P I I E K E R N
 TCT TTG GAA GCC CTG GAA AGC TGC TTT GCT GTC GGC CCA ATC ATT GAG AAG GAG AGA AAC
 2103 2112 2121 2130 2139 2148

 K H A A Q E L A T L L L L S L P A P A S V
 AAG CAC GCA GCC CAG GAG CTG GCC ACT TTG TTG CTC TCC CTG CCA GCG CCT GCC AGC GTC
 2163 2172 2181 2190 2199 2208

 Q Q Q S K S L L A S L H S S R S A Y H S
 CAA CAG CAA TCA AAG AGC CTC CTG GCC AGC CTG CAC AGC AGT CGC TCG GCC TAC CAC AGC
 2223 2232 2241 2250 2259 2268

 H K D Q A L L S K A V Q C L N T S S K E
 CAC AAG GAC CAG GCC TTG TTG AGC AAA GCT GTG CAG TGT CTC AAC ACT TCC AGC AAA GAA
 2283 2292 2301 2310 2319 2328

137/183

FIG.13 I

G	K	D	L	D	P	E	V	F	Q	R	L	V	I	T	A	R	S	I	A	
GGC	AAG	GAC	TTG	GAC	CCC	GAG	GTG	TTC	CAG	CGT	CTA	GTA	ATC	ACA	GCT	CGC	TCT	ATT	GCC	2388
																				2379
																				2370
																				2361
																				2352
																				2343
V	T	R	P	N	N	L	V	H	F	T	E	S	K	L	P	Q	M	E	T	
GTC	ACA	CGT	CCC	AAC	AAC	CTT	GTG	CAC	TTT	ACG	GAG	TCC	AAG	CTG	CCC	CAG	ATG	GAA	ACA	2448
																				2439
																				2430
																				2421
																				2412
																				2403
E	G	A	E	E	G	K	E	P	Q	K	Q	V	E	G	D	G	C	S	F	
GAA	GGA	GCG	GAG	GAG	GGG	AAA	GAG	CCG	CAG	AAG	CAG	GTG	GAA	GGA	GAC	GGC	TGT	AGT	TTC	2508
																				2499
																				2490
																				2481
																				2472
																				2463
I	T	Q	L	V	N	H	F	W	K	L	H	A	S	K	P	K	N	A	F	
ATC	ACT	CAG	CTT	GTA	AAC	CAC	TTC	TGG	AAA	CTC	CAT	GCA	TCT	AAG	CCC	AAG	AAT	GCC	TTC	2568
																				2559
																				2550
																				2541
																				2532
																				2523
L	A	P	A	C	L	P	G	L	T	H	I	E	A	T	V	N	A	L	V	
CTG	GCA	CCT	GCC	TGC	CTG	CCA	GGC	CIT	ACT	CAT	ATT	GAA	GCT	ACG	GTT	AAT	GCG	CTG	GTA	2628
																				2619
																				2610
																				2601
																				2592
																				2583

FIG. 13J

D I I H G Y C T C E L D C I N T A S K I
 GAC ATT ATC CAT GGC TAT TGT ACC TGC GAG CTG GAC TGT ATC AAC ACA GCA TCC AAG ATC
 2643 2652 2661 2670 2679 2688

 Y M Q M L L C P D P A V S F S C K Q A L
 TAC ATG CAG ATG CTG CTG TGT CCT GAC CCT GCT GTG AGC TTC TCC TGT AAA CAA GCT CTA
 2703 2712 2721 2730 2739 2748

 I R V L R P R N K R R R H V T L P S S P R
 ATT CGA GTC CTA AGG CCC AGG AAC AAG CGG AGA CAC GTG ACA TTG CCC TCC TCC CCC CGA
 2763 2772 2781 2790 2799 2808

 S N T P M G D K D D D D D D A D E K M
 AGC AAC ACT CCA ATG GGA GAC AAG GAT GAC GAT GAT GAT GAT GCG GAT GAG AAA ATG
 2823 2832 2841 2850 2859 2868

 Q S S G I P D G G H I R Q Q E S Q E S E
 CAG TCA TCG GGC ATC CCT GAT GGT GGT CAC ATC CGT CAG GAA AGC CAG GAA CAG AGT GAG
 2883 2892 2910 2919 2928

G L S G Q A P S S S L D A G T L S D T
GGA CTG TCG GGC CAG GCA CCC AGC TCT TCC TCT CTG GAC GCA GGA ACC CTC TCT GAC ACC
3183 3192 3201 3210 3219 3228

FIG. 13L

T A S A P A S D D E G S T A A T D G S T
 ACA GCA TCA GCT CCA GCC TCA GAT GAC GAG GGC AGC ACT GCA GCA ACT GAT GGC TCC ACC
 3243 3252 3261 3270 3279 3288

 L R T S P A D H G G S V G S E S G G S A
 CTG CGG ACC TCG CCA GCC GAC CAT GGC GGT AGT GTG GGC TCA GAG AGC GGC GGA AGT GCA
 3303 3312 3321 3330 3339 3348

 V D S V A G E H S V S G R S S A Y G D A
 GTG GAC TCG GTG GCT GGC GAG CAC AGT GTG TCT GGC CGG AGC AGT GCA TAT GGT GAC GCC
 3363 3372 3381 3390 3399 3408

 T A E G H P A G P G S V S S T G A I S
 ACA GCT GAG GGG CAC CCG GCT GGA CCA GGC AGC GTC AGC TCT AGC ACA GGC GCC ATC AGC
 3423 3432 3441 3450 3459 3468

 T T T G H Q E G D G S E G E G E A E
 ACC ACT ACT GGG CAC CAG GAG GGA GAT GGA TCT GAG GGA GAA GGA GAA GCT GAA
 3483 3492 3501 3510 3519 3528

FIG. 13M

G D V H T S N R L H M V R L M L L E R L
 GGA GAT GTG CAC ACT AGC AAC AGG CTA CAC ATG GTT CGT CTA ATG CTG TTG GAA AGA TTA
 3543 3552 3570 3579 3588

 L Q T L P Q L T D L D G E D E K A I P Y M Q
 CTG CAG ACA CTG CCC CAG TTA CGG AAT GTC GGA GGT GTC CGG GCC ATC CCA TAC ATG CAG
 3603 3612 3621 3630 3648

 V I L M L T T D L D G E D E K A A G A L
 GTC ATT CTC ATG CTC ACT ACA GAT CTG GAT GGA GAA GAT GAG AAA GAC AAG GGA GCC CTG
 3663 3672 3681 3690 3708

 D N L L A Q L I A E L G M D K K D V S K
 GAC AAC CTG CTT GCA CAG CTC ATT GCT GAG CTG GGA ATG GAC AAA AAG GAT GTC TCC AAG
 3723 3732 3741 3750 3759 3768

 K N E R S A L N E V H L V V M R L L S V
 AAG AAT GAA CGC AGT GCA TTG AAC GAA GTC CAT TTG GTC GTG ATG AGA CTC CTG AGT GTC
 3783 3792 3801 3810 3819 3828

SUBSTITUTE SHEET (RULE 26)

FIG. 130

E M V L R L P Y Q I K K I A D T S S R I
 GAA A'IG G'IA C'IG AGG C'IT CCC TAC CAG ATC AAG AAG ATC GCA GAT ACC AGC TCT CGA ATC
 4143 4152 4161 4170 4179 4188

 P P P V F D H S W F Y F L S E Y L M I Q
 CCT CCT GTC TTT GAT CAC TCC TGG TTC TAC TTT CTC TCT GAG TAC CTA ATG ATC CAG
 4203 4212 4221 4230 4239 4248

 Q T P F V R R Q V R K L L L L F I C G S K
 CAG ACC CCT TTT GTC CGC CGC CAA GTC CGG AAA CTT CTG CTC TTC ATC TGT GGA TCA AAG
 4263 4272 4281 4290 4299 4308

 E K Y R Q L R D L H T L D S H V R G I K
 GAG AAA TAC CGT CAG CTC CGA GAC CTG CAC ACC CTG GAC TCC CAT GTG CGT GGG ATC AAG
 4323 4332 4341 4350 4359 4368

 K L L E E Q G I F L R A S V V T A S S G
 AAG CTG CTG GAG GAA CAA GGC ATC TTC CTC AGG GCC AGC GTG GTT ACA GCC AGT TCC GGC
 4383 4392 4401 4410 4419 4428

FIG.13P

S A L Q Y D T L I S L M E H L K A C A E
 TCC GCT TTG CAG TAT GAC ACG CTC ATC AGC CTG ATG GAG CAC CTG AAG GCC TGT GCA GAG
 4443 4452 4461 4470 4479 4488

 I A A Q CAG CGC ACC ATC AAC TGG CAG AAG TTC TGC ATC AAA GAT GAC TCT GTC CTG
 ATT GCC GCC CAG 4503 4512 4521 4530 4539 4548

 Y F L L Q V S F L L V D E G V S P V L L Q
 TAC TTC CTC CTG CAA GTG AGT TTC CTG GTA GAT GAG GGG GTG TCC CCT GTG CTA CTG CAG
 4563 4572 4581 4590 4599 4608

 L L S C A L C G S K V L A A L A A S T G
 CTG CTT TCC TGT GCT CTT TGC GGC AGC AAA GTG CTC GCA GCC CTG GCG GCC TCC ACG GGC
 4623 4632 4641 4650 4659 4668

 S S S V A S S A P P A A S S G Q T T T Q
 TCC TCC AGT GTG GCT TCC TCA GCC CCT CCT GCA GCC AGT TCT GGA CAA ACC ACA ACT CAG
 4683 4692 4701 4710 4719 4728

FIG. 13Q

S	K	S	S	T	K	K	S	K	S	K	E	K	E	K	E	G	E		
TCC	AAA	TCC	TCC	ACT	AAA	AAG	AGC	AAG	AAA	AAA	GAA	AAG	GAG	AAA	GAG	GGT	GAG		
	4743			4752		4761		4770		4779		4788							
S	S	G	S	Q	E	D	Q	L	C	T	A	L	V	N	Q	L	N	R	F
AGC	TCG	GGT	AGC	CAG	GAA	GAC	CAG	CTG	TGT	ACA	GCT	CTC	GTG	AAC	CAG	CTG	AAC	AGA	TTT
	4803			4812		4821		4830		4839		4848							
A	D	K	E	T	L	I	Q	F	L	R	C	F	L	L	E	S	N	S	S
GCA	GAC	AAG	GAG	ACT	CTG	ATC	CAG	TTT	CTG	CGC	TGC	TTC	CTA	TTG	GAG	TCC	AAC	TCT	TCG
	4863			4872		4881		4890		4899		4908							
S	V	R	W	Q	A	H	C	L	T	L	H	I	Y	R	N	S	N	K	A
TCA	GTA	CGC	TGG	CAG	GCC	CAC	TGC	CTG	ACA	CTG	CAC	ATC	TAC	AGA	AAC	TCC	AAC	AAG	GCT
	4923			4932		4941		4950		4959		4968							
Q	Q	E	L	L	L	D	L	M	W	S	I	W	P	E	L	P	A	Y	G
CAG	CAG	GAG	CTC	TTG	CTA	GAT	CTC	ATG	TGG	TCA	ATC	TGG	CCA	GAA	CTC	CCA	GCT	TAT	CGT
	4983			4992		5001		5010		5019		5028							

FIG.13R

R K A A Q F V D L L G Y F S L K T A Q T
 CGG AAG GCT GCC CAG TTT GTG GAT CTC CTG GGC TAT TTC TCC CTG AAA ACT GCA CAG ACA
 5043 5052 5061 5070 5079 5088

 E K K L K E Y S Q K A V E I L R T Q N H
 GAG AAG AAG TTG AAG GAG TAC TCA CAG AAG GCC GTG GAG ATT CTG AGG ACT CAG AAC CAC
 5103 5112 5121 5130 5139 5148

 I L T N H P N S N I Y N T L S G L V E F
 ATT CTC ACC AAT CAT CCC AAC TCC AAC ATT TAC AAT ACC TTG TCT GGC TTA GTG GAG TTT
 5163 5172 5181 5190 5199 5208

 D G Y Y L E S D P C L V C N N P E V P F
 GAT GGC TAC TAC CTA GAG AGT GAT CCC TGC CTG GTG TGT AAT AAC CCT GAA GTG CCG TTT
 5223 5232 5241 5250 5259 5268

 C Y I K L S S I I K V D T R Y T T Q Q V
 TGT TAT ATC AAG CTG TCT TCC ATT AAA GTG GAC ACA CGG TAC ACC ACC ACC CAG CAG GTG
 5283 5292 5301 5310 5319 5328

FIG. 13S

V K L I G S H T I S K V T V K I G D L K
 GTA AAG CTC ATT GGT AGT CAC ACC ATC AGC AAG GTG ACA GTG AAA ATT GGG GAC CTG AAG
 5343 5352 5361 5370 5379 5388

 R T K M V R R T I N L Y Y N N R T V Q A I
 CGG ACC AAG ATG GTG CGG ACA ATC AAC CTG TAT TAC AAC AAC CGA ACT GTG CAG GCC ATT
 5403 5412 5421 5430 5439 5448

 V E L K N K P A R W H K A K K V Q L T P
 GTG GAG TTG AAA AAT AAG CCA GCT CGC TGG CAC AAA GCC AAG AAA GTT CAG CTG ACG CCT
 5463 5472 5481 5490 5499 5508

 G Q T E V K I D L P L P I V A S N L M I
 GGA CAG ACA GAA GTC AAG ATT GAC CTG CCT CTG CCC ATT GTG GCC TCT AAC CTG ATG ATC
 5523 5532 5541 5550 5559 5568

 E F A D F Y E N Y Q A S T E T L Q C P R
 GAG TTT GCA GAC TTC TAT GAG AAC TAC CAG GCT TCC ACA GAG ACC CTT CAG TGT CCT CGC
 5583 5592 5601 5610 5619 5628

FIG. 13T

C	S	A	S	V	P	A	N	P	G	V	C	G	N	C	G	E	N	V	Y
TGC	AGT	GCC	TCT	GTC	CCC	GCC	AAT	CCC	GGC	GTC	TGT	GGC	AAC	TGT	GGC	GAG	AAT	GTA	TAC
		5643			5652			5661		5670				5679			5688		
Q	C	H	K	C	R	S	I	N	Y	D	E	K	D	P	F	L	C	N	A
CAG	TGT	CAC	AAG	TGC	AGG	TCC	ATC	AAC	TAT	GAT	GAG	AAG	GAT	CCC	TTC	CTC	TGC	AAT	GCT
		5703			5712			5721		5730				5739			5748		
C	G	F	C	K	Y	A	R	F	D	F	M	L	Y	A	K	P	C	C	P
TGT	GGC	TTT	TGT	AAA	TAT	GCC	CGC	TTT	GAC	TTC	ATG	CTT	TAT	GCC	AAA	CCT	TGC	TGC	CCA
		5763			5772			5781		5790				5799			5808		
V	D	P	I	E	N	E	E	D	R	K	K	A	V	S	N	I	N	T	L
GTG	GAT	CCC	ATC	GAG	AAC	GAG	GAA	GAT	CGG	AAG	AAG	GCC	GTT	TCC	AAC	ATC	AAC	ACA	CTT
		5823			5832			5841		5850				5859			5868		
L	D	K	A	D	R	V	Y	H	Q	L	M	G	H	R	P	Q	L	E	N
CTG	GAC	AAA	GCT	GAC	CGC	GTG	TAC	CAT	CAG	CTC	ATG	GGA	CAC	CGG	CCC	CAG	CTG	GAG	AAC
		5883			5892			5901		5910				5919			5928		

149/183

FIG. 13U

L L C K V N E A A P E K P Q E D S G T A
 CTG CTC TGC AAA GTG AAT GAA GCA GCT CCA GAA AAG CCA CAG GAA GAC TCG GGA ACA GCG
 5943 5952 5961 5970 5979 5988

 G G I S S T S A S V N R Y I L Q L A Q E
 GGA GGC ATC AGC TCC ACT TCA GCC AGT GTG AAT CGC TAC ATC CTA CAG CTG GCG CAG GAG
 6003 6012 6021 6030 6039 6048

 Y C G D C K N S F D E L S K I I Q K V F
 TAT TGT GGA GAC TGT AAG AAC TCA TTT GAC GAG CTC TCC AAA ATC ATC CAG AAA GTC TTC
 6063 6072 6081 6090 6099 6108

 A S R K E L L E Y D L Q Q R E A A T K S
 GCT TCG CGC AAA GAG CTG TTA GAG TAT GAC CTG CAG CAG AGA GAA GCG GCC ACC AAG TCA
 6123 6132 6141 6150 6159 6168

 S R T S V Q P T F T A S Q Y R A L S V L
 TCC CGG ACA TCC GTG CAG CCC ACT GCG TTT ACT GCC AGC CAG TAC CGT GCG TTG TCT GTC TTA
 6183 6192 6201 6210 6219 6228

FIG. 13V

G	C	G	H	T	S	S	T	K	C	Y	G	C	A	S	A	V	T	E	H
GGC	TGT	GGC	CAC	ACC	TCC	TCC	ACC	AAG	TGC	TAT	GGC	TGT	GCC	TCA	GCT	GTC	ACA	GAG	CAT
6243					6252			6261			6270			6279			6288		
C	I	T	L	L	R	A	L	A	T	N	P	A	L	R	H	I	L	V	S
TGT	ATC	ACA	CTG	CTA	AGG	GCC	CTG	GCC	ACC	AAT	CCA	GCC	CTG	AGA	CAC	ATC	CTC	GTC	TCC
6303					6312			6321			6330			6339			6348		
Q	G	L	I	R	E	L	F	D	Y	N	L	R	R	G	A	A	A	I	R
CAG	GGC	CTC	ATC	CGA	GAG	CTC	TTT	GAT	TAC	AAC	CTC	CGC	AGA	GGC	GCG	GCA	GCC	ATC	CGC
6363					6372			6381			6390			6399			6408		
E	E	V	R	Q	L	M	C	L	L	T	R	D	N	P	E	A	T	Q	Q
GAG	GAG	GTC	CGC	CAG	CTC	ATG	TGC	CTC	CTG	ACT	AGA	GAC	AAC	CCA	GAG	GCC	ACC	CAG	CAA
6423					6432			6441			6450			6459			6468		
M	N	D	L	I	I	G	K	V	S	T	A	L	K	G	H	W	A	N	P
ATG	AAT	GAC	CTG	ATC	ATT	GGC	AAA	GTC	TCC	ACT	GCA	CTG	AAG	GGC	CAC	TGG	GCT	AAT	CCT
6483					6492			6501			6510			6519			6528		

151/183

FIG. 13W

D L A S S L Q Y' E M L L L L L T D S I S K E
 GAC CTG GCT AGC AGC CTT CAG TAT GAG ATG CTG CTG CTG ACA GAC TCC ATC TCC AAG GAG
 6543 6552 6561 6570 6579 6588

 D S C W E L R L R C A L S L F L M A V N
 GAC AGC TGC TGG GAA CTC CGC TTA CGC TGT GCA CTC AGC CTT TTC CTC ATG GCT GTG AAC
 6603 6612 6621 6630 6639 6648

 I K T P V V V E N I T L L M C L R I L L Q K
 ATC AAA ACG CCA GTG GTT GTT GAG AAC ATC ACC CTC ATG TGT CTG CGG ATC TTA CAG AAG
 6663 6672 6681 6690 6699 6708

 L I K P P A P T S K K N K D V P V E A L
 CTG ATT AAA CCA CCT GCC CCA ACC AGC AAG AAC AAG GAC GTC CCT GTG GAG GCC CTC
 6723 6732 6741 6750 6759 6768

 T T V K P Y C N E I H A Q A Q Q L W L K R
 ACC ACG GTG AAG CCG TAC TGC AAT GAG ATC CAC GCG CAG GCT CAG CTG TGG CTC AAG CGA
 6783 6792 6801 6810 6819 6828

FIG. 13X

D P K A S Y E A W K K C L P I R G V D G
 GAT CCT AAG GCA TCC TAC GAA GCC TGG AAG AAG TGC CTG CCT ATC CGA GGG GTA GAT GGC
 6843 6852 6861 6870 6879 6888

 N G K S P S K S E L H R L Y L T E K Y V
 AAC GGG AAA TCC CCC AGC AAG TCC GAG CTC CAC CGG CTC TAC TTG ACT GAG AAG TAT GTG
 6903 6912 6921 6930 6939 6948

 W R W K Q F L S R R G G G AAG AGG ACC ACC CCA CTT GAC CTC AAG
 TGG AGG TGG AAA CAG TTC CTG AGT CGG CGG GGG AAG AGG ACC ACC 6999 7008
 6963 6972 6981 6990

 L G H M N W L R Q V L F T P A T Q A A R
 CTG GGC CAC AAC AAC TGG CTG CGG CAG GTA CTC TTT ACC CCG GCA ACA CAG GCA GCA CGG
 7023 7032 7041 7050 7059 7068

 Q A A C T I V E A L A T V P S R K Q Q V
 CAG GCA GCC TGT ACT ATT GTG GAA GCT CTT GCT ACT GTG CCC AGC CGC AAG CAG CAG GTC
 7083 7092 7101 7110 7119 7128

FIG. 13Y

L D L L T S Y L L D E L S V A G E C A A E
 CTT GAC CTC CTC ACC AGT TAC CTG GAC GAG CTG AGT GTG GCT GGG GAG TGT GCT GCA GAG
 7143 7152 7161 7170 7179 7188

 Y L A L Y Q K L I A S C H W K V Y L A A
 TAC CTG GCT CTC TAC CAG AAG CTC ATC GCC TCC TGC CAC TGG AAA GTC TAC CTG GCT GCT
 7203 7212 7221 7230 7239 7248

 R G V L P Y V G N L I T K E I A R L L A
 CGG GGA GTC CTC CCC TAC GTG GGC AAC CTC ATC ACC AAG GAA ATC GCC CGC CTG CTG GCC
 7263 7272 7281 7290 7299 7308

 L E E A T L S T D L Q Q Q G Y A L K S L T
 TTG GAG GAG GCC ACA CTG AGC ACA GAC CTG CAG CAG GGA TAT GCC CTC AAG AGT CTC ACA
 7323 7332 7341 7350 7359 7368

 G L L S S F V E V E S I K R H F K S R L
 GGC CTC TCC TCC TTT GTA GAG GTG GAG TCC ATC AAG CGT CAT TTC AAG AGC CGC TTG
 7383 7392 7401 7410 7419 7428

E	N	E	V	T	E	F	F	V	T	L	E	K	D	P	Q	Q	E	D	F
GAA	AAT	GAA	GTC	ACT	GAG	TTC	TTC	TTT	GTG	ACC	CTG	GAG	AAG	GAC	CCC	CAG	CAA	GAG	GAC
		7683				7692		7701		7710				7719		7728			

155/183

FIG. 13AA

L Q G R M P G N P Y S S N E P G I G P L
 CTA CAG GGC AGG ATG CCC GGA AAC CCA TAT AGC AGC AAT GAA CCA GGC ATT GGG CCT CTT
 7743 7752 7761 7770 7779 7788

 M R D I K N K I C Q D C D L V A L L E D
 ATG AGG GAC ATA AAG AAC AAG ATC TGC CAG GAC TGT GAC CTG GTG GCT CTT CTG GAG GAC
 7803 7812 7821 7830 7839 7848

 D S G M E L L V N N K I I S L D L P V A
 GAC AGT GGG ATG GAG CTT CTA GTG AAC AAT AAA ATT ATC AGT CTG GAT CTT CCT GTG GCT
 7863 7872 7881 7890 7899 7908

 E V Y K K V W C T T N E G E P M R I V Y
 GAG GTT TAC AAG AAG GTC TGG TGT ACG ACC AAT GAG GGA GAG CCC ATG AGG ATT GTT TAT
 7923 7932 7941 7950 7959 7968

 R M R G L L G D A T E E F I E S L D S T
 CGA ATG CGG GGG CTA CTG GGC GAT GCC ACC GAG GAG TTT ATC GAG TCC CTG GAC TCC ACC
 7983 7992 8001 8010 8019 8028

FIG. 13BB

T D E E E D E E E V Y R M A G V M A Q C
 ACA GAT GAA GAG GAC GAA GAG GAA GTG TAC AGA ATG GCC GGT GTG ATG GCC CAG TGC
 8043 8052 8061 8070 8079 8088

 G G L Q C M L N R L A G V K D F K Q G R
 GGG GGT CTG CAG TGC ATG CTG AAC AGA CTG GCA GGA GTC AAA GAT TTT AAG CAG GGG CGC
 8103 8112 8121 8130 8139 8148

 H L L T V L L K L F S Y C V K V K V N R
 CAC CTT CTA ACA GTG CTC CTG AAG CTG TTC AGT TAC TGT GTG AAG GTG AAA GTC AAT CGG
 8163 8172 8181 8190 8199 8208

 Q Q L V K L E M N T L N V M L G T L N L
 CAA CAG TTG GTC AAG CTG GAA ATG AAC ACC TTG AAT GTC ATG TTG GGG ACT TTA AAC TTG
 8223 8232 8241 8250 8259 8268

 A L V A E Q E S K D S G G A A V A E Q V
 GCT CTG GTA GCT GAG CAA GAG AGC AAG GAC GAC GGA GGA GCA GCG GTG GCT GAG CAG GTG
 8283 8292 8301 8310 8319 8328

FIG. 13CC

L S I M E I I L D E S N A E P L S E D K
 CTG AGC ATC ATG GAG ATC ATT CTA GAT GAG TCC AAT GCA GAG CCC CTG AGT GAG GAC AAG
 8343 8352 8361 8370 8379 8388

 G N L L L T G D K D Q L V M L L D Q I N
 GGC AAC CTC CTC ACA GGT GAC AAG GAT CAA CTG GTG ATG CTC TTG GAC CAG ATC AAC
 8403 8412 8421 8430 8439 8448

 S T F V R S N P S V L Q G L L L R I I P Y
 AGC ACC TTC GTT CGT TCC AAT CCC AGC GTG CTG CAG GGT TTG CTG CGT ATC ATC CCA TAC
 8463 8472 8481 8490 8499 8508

 L S F G E V E K M Q I L V E R F K P Y C
 CTG TCC TTC GGA GAG GTG GAG AAG ATG CAG ATC CTG GTG GAG CGG TTC AAG CCA TAC TGC
 8523 8532 8541 8550 8559 8568

 S F D K Y D E D H S G D D K V F L D C F
 AGC TTC GAT AAG TAC GAT GAA GAC CAT AGT GGG GAT GAC AAA GTC TTC CTG GAT TGC TTC
 8583 8592 8601 8610 8619 8628

FIG. 13DD

C	K	I	A	A	G	I	K	N	N	S	N	G	H	Q	L	K	D	L	I	
TGC	AAG	ATT	GCT	GCT	GGT	ATC	AAG	AAC	AAC	AGC	AAT	GGT	CAT	CAG	CTG	AAG	GAC	CTC	ATC	8643
																				8652
																				8661
																				8670
																				8679
																				8688
L	Q	K	G	I	T	Q	S	A	L	D	Y	M	K	K	H	I	P	S	A	
CTG	CAG	AAG	GGA	ATC	ACC	CAG	AGT	GCC	CTG	GAC	TAC	ATG	AAA	AAG	CAT	ATC	CCC	AGT	GCC	8703
																				8712
																				8721
																				8730
																				8739
K	N	L	D	A	D	I	W	K	K	F	L	S	R	P	A	L	P	F	I	
AAG	AAT	TTG	GAT	GCT	GAC	ATC	TGG	AAA	AAG	TTT	TTG	TCT	CGG	CCT	GCT	CTG	CCA	TTT	ATT	8763
																				8772
																				8781
																				8790
																				8799
																				8808
L	R	L	L	R	G	L	A	M	Q	H	P	A	T	Q	V	L	I	G	T	
TTG	AGG	CTT	CTT	CGG	GGT	CTG	GCC	ATG	CAA	CAC	CCT	GCC	ACC	CAG	GTT	CTG	ATT	GGA	ACG	8823
																				8832
																				8841
																				8850
																				8859
																				8868
D	S	I	T	S	L	H	K	L	E	Q	V	S	S	D	E	G	I	G	T	
GAC	TCC	ATC	ACA	AGC	CTG	CAT	AAG	CTA	GAG	CAG	GTG	TCC	AGT	GAT	GAG	GGC	ATT	GGG	ACC	8883
																				8892
																				8910
																				8919
																				8928

159/183

FIG. 13EE

L A E N L L E A L R E H P D V N K K I D
 TTG GCC GAG AAC CTC CTG GAG GCC CTA AGA GAA CAC CCA GAT GTG AAC AAG AAG ATT GAC
 8943 8952 8961 8970 8979 8988

A A R R E T R A E K K R M A M A R Q K
 GCA GCC CGC AGA GAG ACC CGG GCA GAG AAG AAG CGC ATG GCC ATG GCC ATG AGG CAG AAG
 9003 9012 9021 9030 9039 9048

A L G T L G M T T N E K G Q V V T K T A
 GCT CTG GGA ACT CTG GGC ATG ACG ACA AAC GAG AAG GGT CAG GTG GTG ACC AAG ACT GCG
 9063 9072 9081 9090 9099 9108

L L K Q M E E L I E E P G L T C C I C R
 CTC CTG AAG CAA ATG GAA GAG CTG ATT GAG GAG CCC GGA CTC ACG TGC TGC ATC TGC AGG
 9123 9132 9141 9150 9159 9168

E G G T K F Q P T K V L G I Y T F T K R
 GAA GGG GGT ACC AAG TTC CAG CCC ACG AAG GTC TTA GGC ATT TAC ACT TTC ACC AAG CGG
 9183 9192 9201 9210 9219 9228

160/183

FIG. 13FF

V A L E E M E N K P R K Q Q G Y S T V S
 GTG GCC TTG GAG GAG ATG GAG AAC AAG CCT CGG AAA CAG CAG GGC TAC AGC ACT GTG TCC
 9243 9252 9261 9270 9279 9288

H F N I V H Y D C H L A A V R L A R G R
 CAT TTC AAC ATC GTG CAC TAT GAC TGT CAC CTG GCT GCT GTC AGG TTG GCT AGA GGC CGG
 9303 9312 9321 9330 9339 9348

E E W E S A A L Q N A N T K C N G L L P
 GAA GAG TGG GAA AGT GCT GCC CTG CAG AAC GCC AAC ACG AAG TGC AAT GGA CTC CTT CCG
 9363 9372 9381 9390 9399 9408

V W G P H V P E S A F A T C L A R H N T
 GTC TGG GGC CCC CAT GTC CCT GAG TCA GCT TTT GCC ACT TGT TTA GCT AGG CAC AAC ACT
 9423 9432 9441 9450 9459 9468

Y L Q E C T G Q R E P T Y Q L N I H D I
 TAC CTC CAG GAG TGT ACC GGC CAG CGG GAG CCC ACG TAC CAG CTC AAC ATC CAC GAC ATC
 9483 9492 9501 9510 9519 9528

161/183

FIG. 13GG

K L L F L R F A M E Q S F S A D T G G G
 AAA CTG CTT TTC CTG CGC TTC GCC ATG GAG CAG TCA TTC AGT GCA GAC ACC GGT GGG GGC
 9543 9552 9561 9570 9579 9588

G R E S N I H L I P Y I I H T V L Y V L
 GGT CGG GAG AGC AAC ATC CAC CTG ATC CCG TAC ATC ATT CAC ACT GTG CTT TAC GTC CTG
 9603 9612 9621 9630 9639 9648

N T T R A T S R E E K N L Q G F L E Q P
 AAC ACG ACC CGA GCA ACG TCC CGG GAG GAG AAG AAC CTC CAA GGC TTC CTG GAG CAG CCC
 9663 9672 9681 9690 9699 9708

R E K W T E S A F D V D G P H Y F T I L
 AGA GAG AAG TGG ACA GAG AGT GCC TTT GAT GTA GAT GGG CCC CAC TAC TTC ACC ATC TTA
 9723 9732 9741 9750 9759 9768

A L H V L P P E Q W K A T R V E I L R R
 GCC CTG CAT GTC CTC CCC CCT GAG CAG TGG AAA GCC ACT CGA GTG GAG ATC CTA CGC AGG
 9783 9792 9801 9810 9819 9828

FIG. 13HH

L	L	V	A	S	H	A	R	A	V	A	P	G	G	A	T	R	L	T	D
CTG	CTG	GTG	GCC	TCG	CAT	GCC	CGG	GCA	GTG	GCT	CCA	GGA	GGA	GCC	ACC	AGG	TTG	ACA	GAT
9843				9852		9861		9870		9879		9888							
K	A	V	K	D	Y	S	A	Y	R	S	S	L	L	F	W	A	L	V	D
AAG	GCG	GTG	AAA	GAC	TAT	TCT	GCC	TAC	CGT	TCT	TCC	TTG	CTG	TTC	TGG	GCT	CTC	GTT	GAT
9903				9912		9921		9930		9939		9948							
L	I	Y	N	M	F	K	K	V	P	T	S	N	T						
CTC	ATT	TAT	AAC	ATG	TTT	AAG	AAG	GTG	CCC	ACT	AGT	AAC	ACT	TGA	GGG	CGG	GCT	GGT	CCT
9963				9972		9981		9990		9999		10008							
NGC	TCT	CTG	GCT	GAG	TAC	ATT	CCG	CCA	CAA	ATG	ACA	TGC	CCA	TTC	TAC	GAA	GCT	GCC	GAC
10023				10032		10041		10050		10059		10068							
AAA	GCC	CCT	GAA	AAN	CTT	CCA	GGA	GGA	GTT	CAT	GCC	AGT	GGA	GAC	CIT	CTC	AGA	GTT	CCT
10083				10092		10101		10110		10119		10128							

163/183

FIG. 13 II

CGA CGC AGC AGG TCT TCT ATC AGA AAT CAC CGA CCC AGA GAG CTT CCT AAA GGA CCT GTT
 10143 10152 10161 10170 10179 10188

GAA CTC AGT CCC CTG ACC ATC CCC ATG GAA ATG AGA TCG CGG TGA CGA GAT TGA AGC TAG
 10203 10212 10221 10230 10239 10248

CTT GCA TTC TCC CCT CAT TCA TGT CTC CGT GTG CAC TCA TTT CCC CCA CGG ATG CTG CAT
 10263 10272 10281 10290 10299 10308

TAG CAC CCC CTC CTC CCT CAG TTT TCT TGG AGT GGC TTG GGG TTT GTA GGC TTC CTG TTC
 10323 10332 10341 10350 10359 10368

TAT CAC GCG CGC GTG TGT GTG TGT GTG TGT GTG TGT GTG TG
 10383 10392 10401 10410 10419

FIG. 14A

274: CLUSTAL X (1.64b) Multiple Sequence Alignment:

```

274      gi|3413886|dbj|BAA32307|
          gi|4426611|gb|AAD20450|
          MSAHSGGTDWNSVVKALLNRTGALNKNEVVNLLKAITRCEHDFEEESN
          -----
274      gi|3413886|dbj|BAA32307|
          gi|4426611|gb|AAD20450|
          FTQFYTAFAALAADKLMQIKTICQTQICQLHDATAVLIRFIIYRLPRVSV
          -----
274      gi|3413886|dbj|BAA32307|
          gi|4426611|gb|AAD20450|
          YETKWLGLGALKMLCEGRECPASASSMEDYNANAVNLKCKHPSTKSI
          -----
274      gi|3413886|dbj|BAA32307|
          gi|4426611|gb|AAD20450|
          MPSSSSSGSGASNDKESPKSEIKRSRSDLSSVILQQLIAPLEPGKMTWVP
          -----
274      gi|3413886|dbj|BAA32307|
          gi|4426611|gb|AAD20450|
          LSEEVTDCTEQIILAAANVEYFQEQNGVDTLLDVCVSLPILNRYRSKYMETI
          -----

```

274: CLUSTAL X (1.64b) Multiple Sequence Alignment

274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	NGKSLYPLTQVEATAVKSMNHMLTDLTILSOAALIEMQPLTPSRIE
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	RLSMCGIAALYNAVLTSIATSVLGMSQASSSQKTASTSQSGVGSSGG
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	QSNKDHDDFEDQACSI VNKALEIYSNIGHMFKTSARIHVYQNHLCYGSQL
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	LISGIQAMGASGGSSSDSASKSASKATKSGSEAGTAPTTP IARVNLF
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	KVQQGFGEINAAALANHSIKLSELIEDLKVEAACGQSLESTELPEPAQFD

FIG. 14C

274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	ILQNYSSLERIVRLNTATLHQLFTFLATVAYRKACTLKRASAKDRTECE
274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	PISYSDSTTYFNDSLSCSDNSEDDSESYLGHWFKETLSPETHDDNANTS
274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	TQERAEQKSALVPKLDEPHEYLDLAADIFCFLDQFLANRHAYMQRYVVKAG
274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	VSDQOMLLMANIIKDFDRDVMRNETDQSGNAPPAASAGAGTSAGASTKWQ
274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	TSMIRFSGAAGRYIHNLISTSLSEQLSNLLQHLISIPWSTDNTWPLQ
274	gi 3413886 dbj BAA32307	-----	
	gi 4426611 gb AAD20450	-----	VYPSTLSVIVQILLIKPTQEKAAACLSVWHRLINTLVEGVCSSNTASDSD

167/183

FIG. 14D

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274

gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

YEDLNIEHAQLLLFHFSINLMQKKSILLTAGGVIRCAEVCRGISED RP

VKNSQIMLLSRLLLFLEYLMKHLYNAPPELLDQVRWNLFSVSSMPD TQKI

TDLLNCRTKLN SYCRHDIEENFRKSAGEYGSSIRPTFYSLVMGDPE

ISYWAQEFKLDGLAWNFICTPDKLKYPLLVDALTDILSITDMSMY

SMHNLCAIQYCFTIAWKLNGLPPSTSHVESLKAERSPNLHSLMWS

IRLP LASSHYLVVSSLIKQMYTQYAETLWTHVGDIGADIKYSLKQ

FIG. 14E

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

NSQMNGNGTPRLSDLIIFDSLVAHQAVAWANKEGLKWPRKESEDA

AGEQSAGTSLTSSNDPELYSSNESIDDDQKLKQDDDDGKLSSDLQKY

VKLMNSYQLLSVIVRGOMLKQLSSSTPEKALNLIVIPIVSDKPAIML

ELHAAFLKLLPNEDKQLIANEWPKCLMVNDFAFNGKQHPVEPYILN

LTRGSNYSTLHTLKHCLKSILQLFELLPHRTANAETQLKQLLI

SSMLDMRTDYIQGHSEHCLREILSGLTQEAOKLLLYEHMVGYCYRM

FIG. 14F

274
gi|3413886|dbj|EAA32307|
gi|4426611|gb|AAD20450|

RQPQAAGPSGGAPLDQDRAMFNESMLFAVLKTMFKMLEKPVAVQAM

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

RQFFKDQQRSGSLTLLLSFTGTSLPVSYARIKMLQFVNRLLFQLSLQA

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

LVECFSELATVDVARLKQWLGHIIYGPNVSTDVSTSEALDITTCRML

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

TSILQPSSSSSSNAQTPTNMTATVSAMPSISDQLDPMEIEYDCG7AA

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

TGGAANTSQILSLWQAAQDPSEESSQACDHSDSERNGALLLSFV

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

KSLVKDQSKASQIAPPLFQALLQLGOTLISPPQEGCDEFADVLQIMI

FIG. 14G

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

RGHVALFNTTLLWLELAKLQLPDKHLKHAENVSAQLRYLSELLQSI

GFRGSRQHNPPWDDELQTDIDELYDELAEEGEQDSLLDDSDDEDTLN

ALLSKAVQCLNTSS-----KEGKDLDPEVFQRLVITARSIAVTRPNNLV

VLLQFVNRELQSMQPKLEKLETIREIDPEAFYRLVLMVRGIANAR PQSLA

171/183

FIG. 14H

HFTESKLPOMETEGAEKGKEPQKQVEGDGCSFIITQLVNHFWKLGASKPKN

 KICVENN-----YDIVPTILMGIVLELHKVTPTL

AFLAPACLPLGLTHIEATVNALVDIIHGXYCTCELDLCINTASKIYMQMLLCP

 DEPVNIVKRGLCQPETIVHCLVEIMYGFALADPGQVGRMTKYFIDLLKHC

DPAVSFCKQALIRVLRPRNKRHRHVTLPSSPRSNTPMGDKDDDDDDDADE

 ASVISHSAKEALILLSPRMKRRKVAIVTPPACSTPTPSTSTMQALQAAA

274
 gi|3413886|dbj|BAA32307|
 gi|4426611|gb|AAD20450|

274
 gi|3413886|dbj|BAA32307|
 gi|4426611|gb|AAD20450|

274
 gi|3413886|dbj|BAA32307|
 gi|4426611|gb|AAD20450|

FIG. 141

274	gi 3413886 dbj BAA32307	KMQSSGIPDGGHIRQESQEQSEVDHGDFFEMVSESMVLETAENVNNGNPSP
	gi 4426611 gb AAD20450	SSAASDIIEEAAGVVDGVS GGGLPEPNADAEAGAAVGG-VGQQQMLN
274	gi 3413886 dbj BAA32307	RKALLAGAEFPPLMDIPPDADDETMVELAIALSLQQDQSSSALGLQ
	gi 4426611 gb AAD20450	LEAFMGG--GFPRLIGLPEDADDEAIMDIAIALSLQQHGGDANALQSLQQ
274	gi 3413886 dbj BAA32307	SIG-LSGQAPSSSLDAGTSLDTTASAPASDDEGSTAATDGSTLRTSPAD
	gi 4426611 gb AAD20450	GLANLQGIROATAMAAVNAANVSLGGSDDDEGSNVATDGSTLRTSPA
274	gi 3413886 dbj BAA32307	HGGSVGSSEGSADVAGEHSVSGRSSAYGDATAGHPAGPGSVSSS---
	gi 4426611 gb AAD20450	PAGSGSESGSGVESIG---GTSARSSNFGDH-ANASPPRQGSTKDDQE
274	gi 3413886 dbj BAA32307	-TG--AISTTTGHQEGDGEGEAGEGEVHTSNRLHMVRLMLLERLLQ
	gi 4426611 gb AAD20450	QPGPSGVAGSGGVAVLSAMSSSEDNEANEDDKLS-KLHDLRITVLESIIQ
274	gi 3413886 dbj BAA32307	TLPQLRNVGGVRAIPYMQVILMLTTDLDEGEDEKKGALDNLLAQLIAELG
	gi 4426611 gb AAD20450	HLGTFDLCNGLOAIPLIQVILMLTTDLNGNNERDQQVLHDLTALVGLCG

FIG. 14J

274	gi 3413886 dbj BAA32307	MDKDVSKKNE-RSALNEVHLVVMRLLSVFMSRTKS---GSKS---SICE
	gi 4426611 gb AAD20450	MDKDVSKKNE-RSALNEVHLVVMRLLSVFMSRTKS---GSKS---SICE
		NWKGAAARMETKCPGNEVRLALLSLFGVLMGKTKSKQTGTTSPPHQFKD
		*::: *::: *::: *::: *::: *::: *::: *::: *::: *
274	gi 3413886 dbj BAA32307	SSSLISSATAAALLSSGAVDYCLHVLKSLLEYWK-----GQQ
	gi 4426611 gb AAD20450	SSSLISSATAAALLSSGAVDYCLHVLKSLLEYWK-----SQQ
		NSFVASTTANVLSKSGAFVYALEALNTLLVHWKNVLDGDPYAAGGGLASQ
		*::: *::: *::: *::: *::: *::: *::: *::: *
274	gi 3413886 dbj BAA32307	SEEEPVTTS-----QLLKPHTTSPDMSPFLLRQYVKGHAADVFEAYTQL
	gi 4426611 gb AAD20450	NDEEPVATS-----QLLKPHTTSPDMSPFLLRQYVKGHAADVFEAYTQL
		SAQASGGASGPGVQLLKP IKHGP KPD IS ILIPHNYLKNYP-DIFESYDGL
		*::: *::: *::: *::: *::: *::: *::: *::: *
274	gi 3413886 dbj BAA32307	LTEMVLRLPYQIKKIADTSSRI PPPVFDHSWFYFLSEYLMIQQTPEVRRQ
	gi 4426611 gb AAD20450	LTEMVLRLPYQIKKITDTSNRI PPPVFDHSWFYFLSEYLMIQQTPEVRRQ
		LTEIIVRLPYQILRLSSAHPDNYDSGFCEAMFTLCEYMMNLNLTLLRRQ
		*::: *::: *::: *::: *::: *::: *::: *::: *
274	gi 3413886 dbj BAA32307	VRKLLLFICGSKEKYRQLRDLHTLDSHVRGKIKLLEEQGI FLRASVVTAS
	gi 4426611 gb AAD20450	VRKLLLFICGSKEKYRQLRDLHTLDSHVRGKIKLLEEQGI FLRASVVTAS
		VRKLLMYICGSKEKFRMYRDGHS LDAHFRVVKRVCN--IVSSKTGAPYNA
		*::: *::: *::: *::: *::: *::: *::: *::: *
274	gi 3413886 dbj BAA32307	SGSALQYDTLISLMEHLKACAEIAAQRTINWQKFCIKDDSVLYFLQLQVS-
	gi 4426611 gb AAD20450	SGSALQYDTLISLMEHLKACAEIAAQRTINWQKFCIKDDSVLYFLQLQVS-
		NPPMLS YDALVELTEHLRTCQEISQMRTGNWQKFCVVDHEDALAMLMEIAC
		*::: *::: *::: *::: *::: *::: *::: *::: *

FIG. 14K

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
qi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AFD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
qi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

```
FLVDEGVSPVLLQLLSCALCGSKVLAAALAASTGSSSVASS-APPAASSGQ
FLVDEGVSPVLLQLLSCALCGSKVLAAALAASTGSSSVASS-APPAASSGQ
YQLDDGVSPVLLQLLQAACVN-----LPPSGSKQ
*:*****:***.*.*
*:.***:*
```

TTTQSKSSTKKK-K-KEEKEKEGESGSQED-QLCTALVNQLNREADKE
ATTQSKSSTKKK-K-KEEKEKEDGETSGSQED-QLCTALVNQLNKFEADKE
AAQPQPSTSSASGKLRTDREKSEDTDAYYSKFDPACGTFVHQIFRYACDA
. * * . : : : * . : : * . * : : * . : : *

TLIQFLRCFLLESNSSVRWQAHCLTLHIYRNSNKAQQELLDLMLWSIWP
TLIQFLRCFLLESNSSVRWQAHCLTLHIYRNSKSQQELLDLMLWSIWP
LIIRFVRIFLLENNITQLRWQAHSEMTGLFEHANERQREKLNI FWNLWP
*:***: * ****: * :..: *****: : : : : : : * : * : * : * : *

ELPAYGRKAAQFVDLLGYFSLKTAQTEKKLKEYSQAVEILRTQNHLTN
ELPAYGRKAAQFVDLLGYFSLKTPQTEKKLKEYSQAVEILRTQNHLTN
LVPTYGRRRTAQFVDLLGYLTLSRSTRITERLPFVSRAVDVLRQQNELCK

HPNSNIYNTLSGLVEFDGYYLES DPCLVCNNPEVPFCYIKLSSIKVDTRY
HPNSNIYNTLSGLVEFDGYYLES DPCLVCNNPEVPFCYIKLSSIKVDTRY
HPNAPIYTTLIESILQVNGYYLESE PCLVCNNPEVPMANIKLPVS KDSKY
****. ****. ****. ****. ****. ****. ****. ****. ****.

TTTQQVVKLIGSHTISKVTVKIGDLKRTKMVRTINLYNNRRTVQAIVELK
TTTQQVVKLIGSHTISKVTVKIGDLKRTKMVRTINLYNNRRTVQAIVELK
TTTTMIYKLVQCHTISKLIIVRIADLKRTKMVRTINVYNNRSVQAVVELK
: ** . **: *:* . *****: *****: *****: *****

FIG. 14L

NKP PAR WHK AKV QLT PGQ TE VK ID LPL P IVAS N LMI EFAD F YEN YQASTE
 NKP PAR WHK AKV QLT PGQ TE VK ID LPL P IVAS N LMI EFAD F YEN YQASTE
 NRP PAL WHK ARSVLS QA TEL KID F LPL P I TAC N LMI EFAD F FET VSGSSE
 * * * * * * * * * * * * * * * *

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

TTLQPCRSASVPANPGVCGNGENVYQCHKCRSINYDEKDPFLCNACGFC
TTLQPCRSASVPANPGVCGNGENVYQCHKCRSINYDEKDPFLCNACGFC
NLQPCRSAAPVAYPGVCGNGENVFQCHKCRAINYDEKDPFLCHSCGFC

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

KYARFDFMLYAKPCCPVDP IENEEDRKAVSNINTLLDKADRVYHQIMGH
KYARFDFMLYAKPCCAVDP IENEEDRKAVSNINTLLDKADRVYHQIMGH
KYAKFDFSMYARVCCAVDP IESAEDRVKTSLIHSSLERADRNYRQLLTN
***** : ** . ***** *** * : * : * : * : *

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

RPQLNLLCKVNEAPEKQEDSGTAGGTSSTASVNRYYILQLAQEYCGD
RPQLNLLCKVNEAPEKQDDSGTAGGTSSTASVNRYYILQLAQEYCGD
KQMLLELLIQKVAEHRSDRMVEDNMAS-VHSTS-QVNKIIQLLAQKCYVE

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

RALSVLGCGHTSSTKCYGCASAVTEHCITLLRALATNPALRHILVSQGLI
 RALSVLGCGHTSSTKCYGCASAVTEHCITLLRALATNPALRHILVSQGLI
 ---SSTGAENPTTNRCYGCALASTEQCCLTLRAMAYNYDCRVCLYSQGLV
 * * : : : : : * * * * : : * * * * : * * * * : * * * * :

[illegible]

FIG. 14N

[illegible]

FIG. 140

274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	NVRMNSGDQGTIRQLI SAHVLRVAMCVLSSPHGRRQHLAVSHEKGI ----- NVRMNSGDQGTIKHLLSSGVVRRVAFCCLSPPHGRRQQLAVSHEKGV
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	TVLQLSALLKQADSSKRKLTLTRLASAPVFTVLSTGNPCKEDYLVVCG ----- TILQLSALLKQADASKRKLTILTQLSSAPIACTVISLAANPCNEDCLAVCG
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	LKDCHVLTFSSSGSVSDHLVLHPQLATGNFIIKAVWLPGSQTELAIVTAD ----- LKECHILTFSSSGSTNEHIVVNPQLENGNYIKKAVWLPGSQTLAVVTSD
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	FVKIYDLSVDALSPTFYFLLPSSKIRDVTFLFNEEGKNIIVIMSSAGYMY ----- YVKIYDLAVDTYSPKYIYLAVGKIRDCTFVYQDGNYNMLTFASS-GYIY
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	TQLMEEASSAQQGFYVTNVLEINHEDLKDSNSQVAGGVSYYSHVLQM ----- TQQLDQOSLAVHGDFYVTNTLELSHQHIKDIAGHIGGGGVSIIYSHTLQL
274	gi 3413886 dbj BAA32307 gi 4426611 gb AAD20450	IFFSYSQKSFATVSRSTLEVQLFPINIKSSNGGSKTSP--ALCQWSE ----- LFFSYS CGRSFFSPLTNVSEGVKGIYHLDNTNSASKSGPLQPLVQWTE

FIG. 14P

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

VNHPGLVCCVQQTGGVPLVVMVKPDTFLIQEIKTLPAKAKIQDMVAIRH

VTGHPGLVYASMQTSNNPIILMITPERIYLQEIKAQSAKSIRIMDVVGIRH

TACNEQORTTMILLCEDGSLRIYMANVENTSYWLQPSLPSS---VISI

AVAG-VEKTTLLLLCEDGSLRIFSAQPEYTSFWLSPQVQPFQGNLYSSTL

MKP-----VRKRKTATITARTSS-----QVTFPIDFFEHN

MAKGGSGSTSKSNTASGKMTSRKASQQKQKPTAGGQPVFPIDFFEH

QQLTDVEFGNDLLQVYNAQQIKHRLNSTGMYVANTKPGGFTMEISNNSS

NMLADVEFGNDLLQIYNKQKLKTRLFSTGMFVASTRSNGFTLEVINNDP

TMVMTGMRIQIGTQAIERAPSYIEIFGRTMQLNLSRWFDFPFTREEAL

NVVIVGIRVLIGTDVQVQAPQSVTILGRTIPTVRRARWFEDIPLTREML

QADRKLNLEFIGASVDPAGVTMIDAVKIYCKTKEQFGWPDPEPPPSASV

QSDKLLKVVFAKAPDPEHVTLLDCIEVYGKSKELVGWPDESED-----VTV

[illegible]

FIG. 14R

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

274

gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

RLAGVKDFKQGRHLLTVLLKLFSCVVKVNRQQLVKLEMTNLVNLGTL
RLAGIRDFKQGRHLLTVLLKLFSCVVKVNRQQLVKLEMTNLVNLGTL
RIGSLQIRSRNRELIVLLKLFICVKVRRCCQEVLCQPEIGAINTLLKVL
*::: *::: ***** *::: *::: *::: *::: *

NLAIVAEQESKDSGGAAVAEQVLSIMEIILDESNAEPLSEDKGNLLLTGD
NLAIVAEQESKDSGGAAVAEQVLSIMEIILDESNAEPLSEDKGNLLLTGD
QMCLOSENDSIQS---AVTEQLLEIMETILSKAASDTLDSFLQFSLTFGG
*::: *::: *::: ***** *::: *::: *::: *::: *

KDQLVMLLDQINSTFVRSNP SVLQGLLRIPYLSFGEVEKMQILVERFKP
KDQLVMLLDQINSTFVRSNP SVLQGLLRIPYLSFGEVEKMQILVERFKP
PEYVSALISCTDCPNVRNPNP SVLRHLIRVLAALVYGVNEVKMALLCEHFKE
*::: *::: *::: ***** *::: *::: *::: *::: *

YCSFDKYDEDSGDDKVFLLDCFCCKIAAGIKNNSNGHQLKDLILQKGITQS
YCNFDKYDEDSGDDKVFLLDCFCCKIAAGIKNNSNGHQLKDLILQKGITQN
TLNFRNFDNERTPEEEFKLELFCVLTNQIEHNICIGGTLKDYIVSLGIVER
*::: *::: *::: *::: *::: *::: *::: *::: *

ALDXYMKKHIPSAK-----NLDADIWKKFLSRPALPFILRLRLGLAMQHPAT
ALDXYMKKHIPSAK-----NLDADIWKKFLSRPALPFILRLRLGLAIQHPGT
SLAYITEHAPCVKPTLLRTDSDDELKEFISRPSLKYILRFLTGLSNHHEAT
*::: *::: *::: *::: *::: *::: *::: *::: *

QVLIGTDSITSILHKLEQVSSDEGIGTIAENLLEALREHPDVNKKIDAARR
QVLIGTDSIPNLHKLEQVSSDEGIGTIAENLLEALREHPDVNKKIDAARR
QVAISKDIPIIHRLEQVSSDEHVGSLAENLLEALSTDSATAARVQVVRD
** *::: *::: *::: *::: *::: *::: *::: *::: *

274
gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274
gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274
gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

274
gi | 3413886 | dbj | BAA32307 |
gi | 4426611 | gb | AAD20450 |

FIG. 147

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

PHYFTIALHVLPPPEQWKATRV EILRRLLVASHARAVAPGGATR-----LT
PYFTVLALHILPPEQWRATRV EILRRLLVTSQARAVAPGGATR-----LT
PLFMATISLSLHSRELWNKHLAHLKRMIAVAQGRHVSPAVLCKALLAPA
* ::: **: . * * . :: *: : : : : * * * . : :

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

DKAVKDYSAYRSLLFWALVDLIYN-MFKKVPTSNT-----
DKAVKDYSAYRSLLFWALVDLIYN-MFKKVPTSNTTEGGWSCSLAEYIRH
DRQVKDYTVYKPFMMWALVDLIYDNLEKTVSTPKEED-WPISLFDYLRK
*: ****: *: . *: : *****: : ** . * . *: :

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

NDMPIYEAADKALKTFQEEFMPVETFEFLDVAGLLSEITDPESFLKDLL
NDEALLKSTDSILOTLTEEFPCTSFVEEFCDVAGLLHLHIEHPDNFIEEIL

274
gi|3413886|dbj|BAA32307|
gi|4426611|gb|AAD20450|

NSVP-----
AALPSTSSSN

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/11066

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C 12Q 1/68

US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 435/7.1, 514/44, 435/69.1, 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

US PAT, Biosis, embase, caplus, medline, lifesci.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,882,925 A (FALB) 16 March 1999 see entire document.	1-12
Y,P	US 5,861,249 A (BEACH et al) 19 January 1999 see entire.	5, 9-12
Y	US 5,445, 941 A (YANG) 29 August 1995 see entire document.	1-12
Y	US 5,599,708 A (MUNDY et al) 04 February 1997 see entire document.	1-12
Y,P	US 5,763,416 A (BONADIO et al) 09 June 1998 see entire document.	1-12

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 SEPTEMBER 1999

Date of mailing of the international search report

29 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ANNA B. BAWOL

Telephone No. (703) 308-0254

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11066

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/11066

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-12 and 43, drawn to method or process for identifying genes whose expression is responsive to a specific cue or cues (claims 1-12) and a method for advancing research in or studies of bone development (claim 43).
Group II, claim(s) 16-19, drawn to method for determining risk of developing osteoporosis or low or high bone density or other factors causing or contributing to osteoporosis or lower levels of osteoblasts and chondrocytes or other conditions involving mechanical stress or lack thereof.

Group III, claim(s) 19, drawn to method for testing a medicament for or gene therapy approach to osteoporosis.
Group IV, claim(s) 20, 36-40, 44 and 45, drawn to method of treating, preventing or controlling osteoporosis.

Group V, claim(s) 22, drawn to a cellular-based model of osteoporosis or mechanical stress, or lack thereof.

Group VI, claim(s) 23-28, drawn to nucleic acid, probes, primers (claims 23-25 and 28) and to a vector (claims 26 and 27).

Group VII, claim(s) 29-34 and 41-42, drawn to expression product (polypeptide), composition comprising same, and related method of making same.

Group VIII, claim(s) 34 and 35, drawn to antibody and related composition.

Claim 21 is considered to be generic to the inventions of groups VI, VII, and VIII. Accordingly, it will be searched with any or all of the Groups depending upon the payment of additional fees for having searches conducted for each of said groups.

Election of Species

In the event that applicant elects to pay additional fees for the search of inventions corresponding to Groups III, IV, VI, VII, and VIII, the following election of species will apply.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

- | | |
|-------------|--|
| Claim 21: | a) gene or portion thereof;
b) protein or portion thereof; and
c) antibody or portion thereof. |
| Group IV: | a) protein 274; and
b) protein 405. |
| Group VI: | a) nucleic acid that encodes human protein 608;
b) nucleic acid that encodes human protein 405;
c) nucleic acid that encodes human protein 274; and
d) probe or primer that specifically hybridizes to
i) a);
ii) b); and
iii) c). |
| Group VII: | a) protein 274;
b) protein 405; and
c) protein 608. |
| Group VIII: | a) antibody that is elicited by protein 274;
b) antibody elicited by protein 405; and
c) antibody elicited by protein 608. |

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The inventions are directed to different methods and compounds. The methods comprised of different methods steps, requiring different reagents and resulting in different end products. The compounds are not related by a special technical feature, e.g., the probe/primer need not encode any amino acid sequence nor antibody and can also hybridize specifically to other

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/11066

sequences with equal affinity.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The species are drawn to different proteins, nucleic acids, probes/primers, and antibodies which each have different physical composition and activities.

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.